



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/US99/27948 <b>(22) International Filing Date:</b> 17 December 1999 (17.12.99)  <b>(30) Priority Data:</b> 60/113,955                      23 December 1998 (23.12.98)      US 60/142,603                      7 July 1999 (07.07.99)              US  <b>(71) Applicant (for all designated States except US):</b> G.D. SEARLE & CO. [US/US]; Corporate Patent Dept., P.O. Box 5110, Chicago, IL 60680-5110 (US).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> KELLER, Bradley, T. [US/US]; 1780 Canyon View Court, Chesterfield, MO 63017 (US). GLENN, Kevin, C. [US/US]; 509 Princeton Gate Court, Chesterfield, MO 63017 (US). SCHUH, Joseph, R. [US/US]; 2055 Rurline Drive, St. Louis, MO 63146 (US).  <b>(74) Agents:</b> WILLIAM, Roger, A. et al.; G.D. Searle & Co., Corporate Patent Dept., P.O. Box 5110, Chicago, IL 60680-5110 (US).		<b>(81) Designated States:</b> AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> COMBINATIONS OF ILEAL BILE ACID TRANSPORT INHIBITORS AND FIBRIC ACID DERIVATIVES FOR CARDIOVASCULAR INDICATIONS		
<b>(57) Abstract</b> <p>The present invention provides combinations of cardiovascular therapeutic compounds for the prophylaxis or treatment of cardiovascular disease including hypercholesterolemia, atherosclerosis, or hyperlipidemia. Combinations disclosed include an ileal bile acid transport inhibitor combined with a fibric acid derivative.</p>		

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Combinations of Ileal Bile Acid Transport Inhibitors and  
Fibric Acid Derivatives for Cardiovascular Indications

This application claims priority of U.S. provisional  
5 application Ser. No. 60/142,603 filed Jul. 7, 1999 and of  
U.S. provisional application Ser. No. 60/113,955 filed  
Dec. 23, 1998.

BACKGROUND OF THE INVENTION

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Field of the Invention

The present invention relates to methods of treating  
cardiovascular diseases, and specifically relates to  
combinations of compounds, compositions, and methods for  
15 their use in medicine, particularly in the prophylaxis and  
treatment of hyperlipidemic conditions such as are  
associated with atherosclerosis, hypercholesterolemia, and  
other coronary artery disease in mammals. More  
particularly, the invention relates to ileal bile acid  
20 transporter (IBAT) inhibiting compounds. The invention  
also relates to fibric acid derivatives (fibrates).

Description of Related Art

It is well-settled that hyperlipidemic conditions  
25 associated with elevated concentrations of total  
cholesterol and low-density lipoprotein (LDL)  
cholesterol are major risk factors for coronary heart  
disease and particularly atherosclerosis. Since high  
levels of LDL cholesterol increase the risk of  
30 atherosclerosis, methods for lowering plasma LDL  
cholesterol would be therapeutically beneficial for the  
treatment of atherosclerosis and other diseases  
associated with accumulation of lipid in the blood  
vessels. These diseases include, but are not limited

to, coronary heart disease, peripheral vascular disease, and stroke.

Atherosclerosis underlies most coronary artery disease (CAD), a major cause of morbidity and mortality in modern society. High LDL cholesterol (above about 180 mg/dl) and low HDL cholesterol (below 35 mg/dl) have been shown to be important contributors to the development of atherosclerosis. Other diseases or risk factors, such as peripheral vascular disease, stroke, and hypercholesterolaemia are negatively affected by adverse HDL/LDL ratios.

Interfering with the recirculation of bile acids from the lumen of the intestinal tract is found to reduce the levels of serum cholesterol in a causal relationship. Epidemiological data has accumulated which indicates such reduction leads to an improvement in the disease state of atherosclerosis. Stedronsky, in "Interaction of bile acids and cholesterol with nonsystemic agents having hypocholesterolemic properties," Biochimica et Biophysica Acta, 1210, 255-287 (1994) discusses the biochemistry, physiology and known active agents surrounding bile acids and cholesterol.

Transient pathophysiologic alterations are shown to be consistent with interruption of the enterohepatic circulation of bile acids in humans with an inherited lack of IBAT activity, as reported by Heubi, J.E., et al. See "Primary Bile Acid Malabsorption: Defective in Vitro Ileal Active Bile Acid Transport", Gastroenterology, 83, 804-11 (1982).

In another approach to the reduction of recirculation of bile acids, the ileal bile acid transport system is a putative pharmaceutical target for the treatment of hypercholesterolemia based on an interruption of the enterohepatic circulation with specific transport

inhibitors (Kramer, et al., "Intestinal Bile Acid Absorption" The Journal of Biological Chemistry, 268 (24), 18035-46 (1993).

In several individual patent applications, Hoechst Aktiengesellschaft discloses polymers of various naturally occurring constituents of the enterohepatic circulation system and their derivatives, including bile acid, which inhibit the physiological bile acid transport with the goal of reducing the LDL cholesterol level sufficiently to be effective as pharmaceuticals and, in particular for use as hypocholesterolemic agents. The individual Hoechst patent applications which disclose such bile acid transport inhibiting compounds are each separately listed below.

15

R1. Canadian Patent Application No. 2,025,294.

R2. Canadian Patent Application No. 2,078,588.

R3. Canadian Patent Application No. 2,085,782.

~~R4. Canadian Patent Application No. 2,085,830.~~

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R5. EP Application No. 0 379 161.

R6. EP Application No. 0 549 967.

R7. EP Application No. 0 559 064.

R8. EP Application No. 0 563 731.

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Selected benzothiepinines are disclosed in world patent application number WO 93/321146 for numerous uses including fatty acid metabolism and coronary vascular diseases.

30

Other selected benzothiepinines are known for use as hypolipaemic and hypocholesterolaemic agents, especially for the treatment or prevention of atherosclerosis as disclosed in application No. EP 508425. A French patent application, FR 2661676 discloses additional benzothiepinines for use as hypolipaemic and

hypcholesterolaemic agents. Furthermore, patent application no. WO 92/18462 lists other benzothiepies for use as hypolipaemic and hypcholesterolaemic agents. U.S. Patent No. 5,994,391 (Lee et al.) Each of the

5 benzothiepie hypolipaemic and hypcholesterolaemic agents described in these individual patent applications is limited by an amide bonded to the carbon adjacent the phenyl ring of the fused bicyclobenzothiepie ring.

Further benzothiepies useful for the treatment of

10 hypercholesterolemia and hyperlipidemia are disclosed in patent application no. PCT/US95/10863. More benzothiepies useful for the prophylaxis and treatment of hypercholesterolemia and hyperlipidemia as well as pharmaceutical compositions of such benzothiepies are

15 described in PCT/US97/04076. Still further benzothiepies and compositions thereof useful for the prophylaxis and treatment of hypercholesterolemia and hyperlipidemia are described in U.S. Application Serial No. 08/816,065.

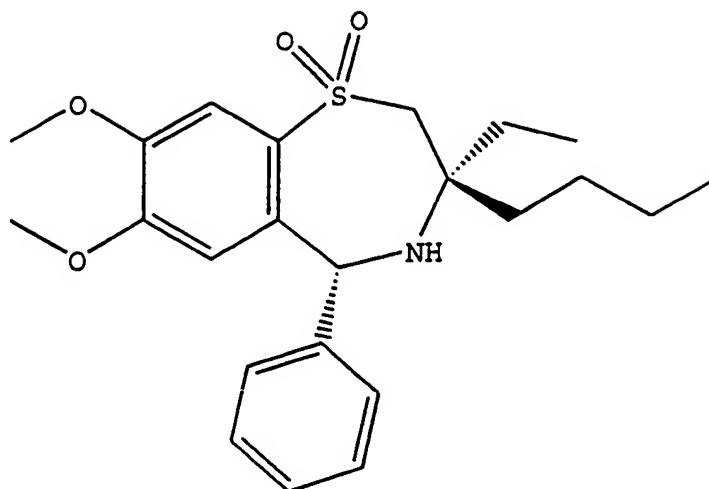
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~~In vitro bile acid transport inhibition is disclosed~~

20 to correlate with hypolipidemic activity in The Wellcome Foundation Limited disclosure of the Patent Application No. WO 93/16055 for "Hypolipidemic Benzothiazepine Compounds." That publication describes a number of hypolipidemic benzothiazepine compounds. Additional

25 hypolipidemic benzothiazepine compounds (particularly 2,3,4,5-tetrahydrobenzo-1-thi-4-azepine compounds) are disclosed in Patent Application No. WO 96/05188. A particularly useful benzothiazepine disclosed in WO 96/05188 is the compound of formula B-2. Further

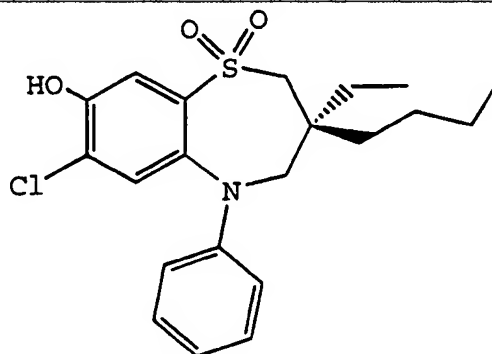
30 hypolipidemic benzothiazepine compounds are described in Patent Application No. WO 96/16051.



B-2

(3R,5R)-3-butyl-3-ethyl-2,3,4,5-tetrahydro-  
7,8-dimethoxy-5-phenyl-1,4-benzothiazepine  
1,1-dioxide

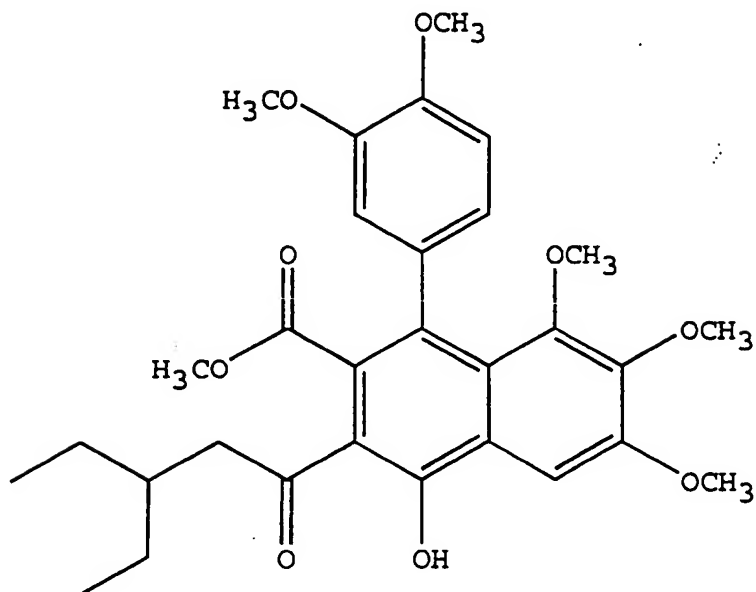
Other benzothiazepine compounds useful for control of cholesterol are described in PCT Patent Application No. WO 99/35135. Included in that description is the compound of formula B-7.



B-7

Further IBAT inhibitor compounds include a class of naphthalene compounds, described by T. Ichihashi et al. in J. Pharmacol. Exp. Ther., 284(1), 43-50 (1998). In this class, S-8921 (methyl 1-(3,4-dimethoxyphenyl)-3-(3-ethylvaleryl)-4-hydroxy-6,7,8-trimethoxy-2-naphthoate) is particularly useful. The structure of S-8921 is shown in formula B-20. Further naphthalene compounds or lignin derivatives useful for the treatment or prophylaxis of

hyperlipidemia or atherosclerosis are described in PCT  
Patent Application No. WO 94/24087.



B-20

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Fibric acid derivatives comprise another class of drugs which have effects on lipoprotein levels. Among the first of these to be developed was clofibrate, disclosed in U.S. patent no. 3,262,850, herein incorporated by

reference. Clofibrate is the ethyl ester of p-chlorophenoxyisobutyric acid. A widely used drug in this class is gemfibrozil, disclosed in U.S. patent no. 3,674,836, herein incorporated by reference. Gemfibrozil frequently is used to decrease triglyceride levels or increase HDL cholesterol concentrations (The Pharmacological Basis of Therapeutics, p. 893).

Fenofibrate (U.S. patent no. 4,058,552) has an effect similar to that of gemfibrozil, but additionally decreases LDL levels. Ciprofibrate (U.S. patent no. 3,948,973) has similar effects to that of fenofibrate. Another drug in this class is bezafibrate (U.S. patent no. 3,781,328). Warnings of side effects from use of fibric acid derivatives include gall bladder disease (cholelithiasis), rhabdomyolysis, and acute renal failure. Some of these



effects are exacerbated when fibrates are combined with HMG CoA reductase inhibitors due to their combined effects on the liver.

Some combination therapies for the treatment of cardiovascular disease have been described in the literature. Combinations of IBAT inhibitors with HMG CoA reductase inhibitors useful for the treatment of cardiovascular disease are disclosed in U.S. Patent Application No. 09/037,308.

10 A combination therapy of fluvastatin and niceritrol is described by J. Sasaki et al. (Id.). Those researchers conclude that the combination of fluvastatin with niceritrol "at a dose of 750 mg/day dose does not appear to augment or attenuate beneficial effects of  
15 fluvastatin."

L. Cashin-Hemphill et al. (J. Am. Med. Assoc., 264 (23), 3013-17 (1990)) describe beneficial effects of a combination therapy of colestipol and niacin on coronary atherosclerosis. The described effects include  
20 nonprogression and regression in native coronary artery lesions.

A combination therapy of acipimox and simvastatin shows beneficial HDL effects in patients having high triglyceride levels (N. Hoogerbrugge et al., J. Internal  
25 Med., 241, 151-55 (1997)).

Sitostanol ester margarine and pravastatin combination therapy is described by H. Gylling et al. (J. Lipid Res., 37, 1776-85 (1996)). That therapy is reported to simultaneously inhibit cholesterol absorption and lower  
30 LDL cholesterol significantly in non-insulin-dependent diabetic men.

Brown et al. (New Eng. J. Med., 323 (19), 1289-1339 (1990)) describe a combination therapy of lovastatin and colestipol which reduces atherosclerotic lesion

progression and increase lesion regression relative to lovastatin alone.

Buch et al. (PCT Patent Application No. WO 9911263) describe a combination therapy comprising amlodipine and a statin compound for treating subjects suffering from  
5 angina pectoris, atherosclerosis, combined hypertension and hyperlipidemia, and to treat symptoms of cardiac arrest. Buch et al. describe in PCT Patent Application No. WO 9911259 a combination therapy comprising amlodipine  
10 and atorvastatin.

Scott et al. (PCT Patent Application No. WO 9911260) describe a combination therapy comprising atorvastatin and an antihypertensive agent.

Dettmar and Gibson (UK Patent Application No. GB  
15 2329334 A) claim a therapeutic composition useful for reducing plasma low density lipoprotein and cholesterol levels, wherein the composition comprises an HMG CoA reductase inhibitor and a bile complexing agent.

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The above references show continuing need to find  
20 safe, effective agents for the prophylaxis or treatment of cardiovascular diseases.

#### Summary of the Invention

To address the continuing need to find safe and  
25 effective agents for the prophylaxis and treatment of cardiovascular diseases, combination therapies of cardiovascular drugs are now reported.

Among its several embodiments, the present invention provides a combination therapy comprising the use of a  
30 first amount of an IBAT inhibitor and a second amount of another cardiovascular therapeutic useful in the prophylaxis or treatment of hyperlipidemia, atherosclerosis, or hypercholesterolemia, wherein said first and second amounts together comprise an anti-

hyperlipidemic condition effective amount, an anti-atherosclerotic condition effective amount, or an anti-hypercholesterolemic condition effective amount of the compounds. For example one of the many embodiments of the present invention is a combination therapy comprising therapeutic dosages of an IBAT inhibitor and a fibric acid derivative. A preferred embodiment of the present invention is a combination therapy comprising therapeutic dosages of a benzothiepine IBAT inhibitor and a fibric acid derivative.

A further embodiment of the instant invention comprises the use of any of the cardiovascular combination therapies described herein for the prophylaxis or treatment of hypercholesterolemia, atherosclerosis, or hyperlipidemia. Therefore, in one embodiment the present invention provides a method for the prophylaxis or treatment of a hyperlipidemic condition comprising administering to a patient in need thereof a combination in unit dosage form wherein the combination comprises a first amount of an ileal bile acid transport inhibiting compound and a second amount of a fibric acid derivative compound wherein the first amount and the second amount together comprise an anti-hyperlipidemic condition effective amount of the compounds.

In another embodiment, the present invention provides a method for the prophylaxis or treatment of an atherosclerotic condition comprising administering to a patient in need thereof a combination in unit dosage form wherein the combination comprises a first amount of an ileal bile acid transport inhibiting compound and a second amount of a fibric acid derivative compound wherein the first amount and the second amount together comprise an anti-atherosclerotic condition effective amount of the compounds.

In still another embodiment, the present invention provides method for the prophylaxis or treatment of hypercholesterolemia comprising administering to a patient in need thereof a combination in unit dosage form wherein  
5 the combination comprises a first amount of an ileal bile acid transport inhibiting compound and a second amount of a fibric acid derivative compound wherein the first amount and the second amount together comprise an anti-hypercholesterolemic condition effective amount of the  
10 compounds.

Further scope of the applicability of the present invention will become apparent from the detailed description provided below. However, it should be understood that the following detailed description and  
15 examples, while indicating preferred embodiments of the invention, are given by way of illustration only since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

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#### DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The following detailed description is provided to aid those skilled in the art in practicing the present  
25 invention. Even so, this detailed description should not be construed to unduly limit the present invention as modifications and variations in the embodiments discussed herein can be made by those of ordinary skill in the art without departing from the spirit or scope of the present  
30 inventive discovery.

The contents of each of the references cited herein, including the contents of the references cited within these primary references, are herein incorporated by reference in their entirety.

a. Definitions

The following definitions are provided in order to aid the reader in understanding the detailed description of the present invention:

"Ileal bile acid transporter" or "IBAT" is synonymous with apical sodium co-dependent bile acid transporter, or ASBT.

"Benzothiepine IBAT inhibitor" means an ileal bile acid transport inhibitor which comprises a therapeutic compound comprising a 2,3,4,5-tetrahydro-1-benzothiepine 1,1-dioxide structure.

"Combination therapy" means the administration of two or more therapeutic agents to treat a hyperlipidemic condition, for example atherosclerosis and hypercholesterolemia. Such administration encompasses co-administration of these therapeutic agents in a substantially simultaneous manner, such as in a single dosage form having a fixed ratio of active ingredients or in multiple, separate dosage forms for each inhibitor agent. In addition, such administration also encompasses use of each type of therapeutic agent in a sequential manner. In either case, the treatment regimen will provide beneficial effects of the drug combination in treating the hyperlipidemic condition.

The phrase "therapeutically effective" is intended to qualify the combined amount of inhibitors in the combination therapy. This combined amount will achieve the goal of reducing or eliminating the hyperlipidemic condition.

"Therapeutic compound" means a compound useful in the prophylaxis or treatment of a hyperlipidemic condition, including atherosclerosis and hypercholesterolemia.

b. Combinations

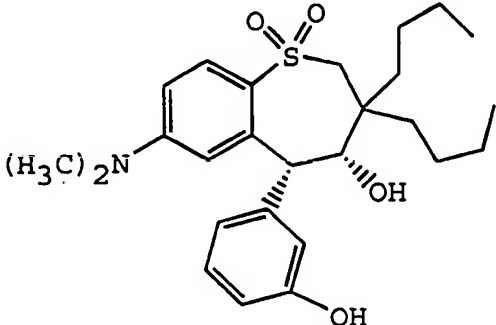
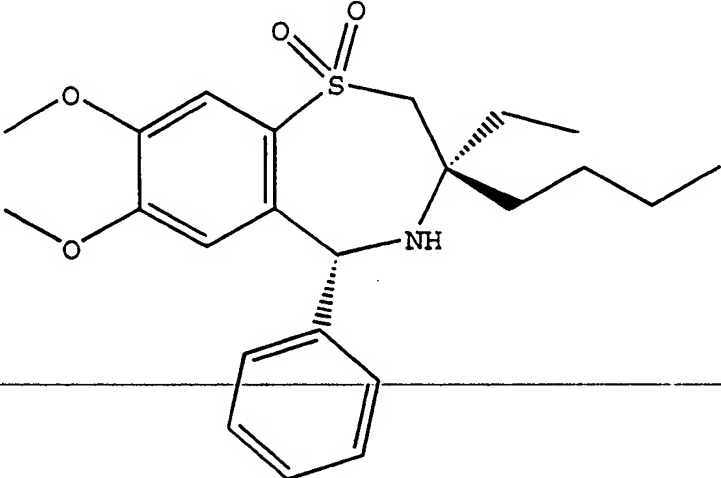
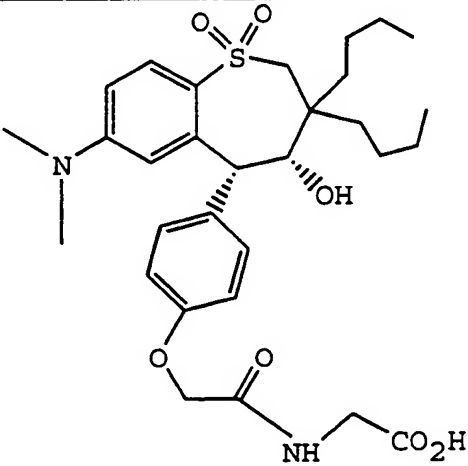
The combinations of the present invention will have a number of uses. For example, through dosage adjustment  
5 and medical monitoring, the individual dosages of the therapeutic compounds used in the combinations of the present invention will be lower than are typical for dosages of the therapeutic compounds when used in monotherapy. The dosage lowering will provide advantages  
10 including reduction of side effects of the individual therapeutic compounds when compared to the monotherapy. In addition, fewer side effects of the combination therapy compared with the monotherapies will lead to greater patient compliance with therapy regimens.

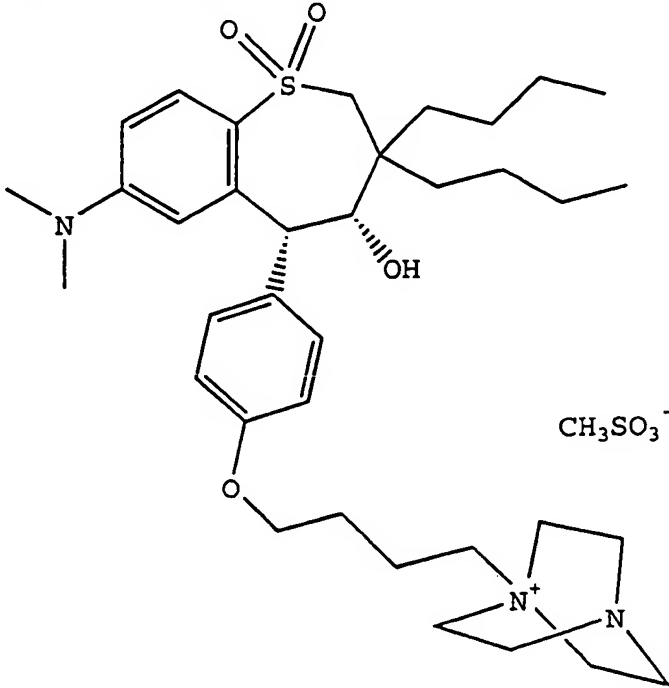
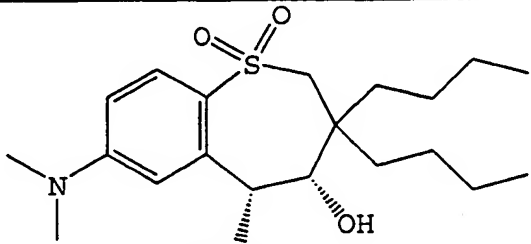
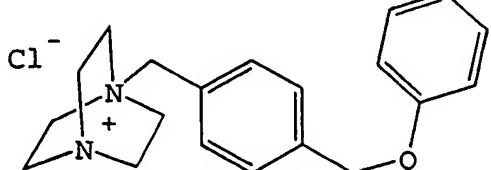
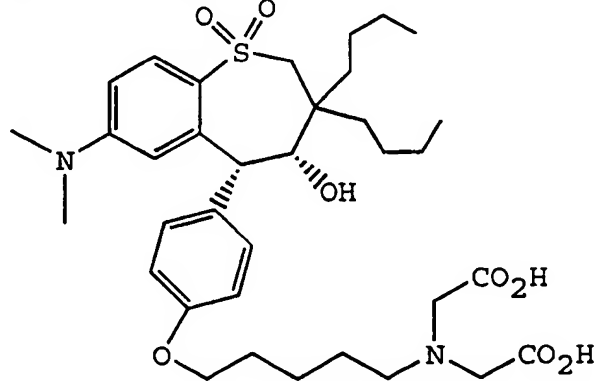
15 Compounds useful in the present invention encompass a wide range of therapeutic compounds. Some IBAT inhibitors useful in the present invention are disclosed in patent application no. PCT/US95/10863, herein incorporated by reference. ~~More IBAT inhibitors are described in~~

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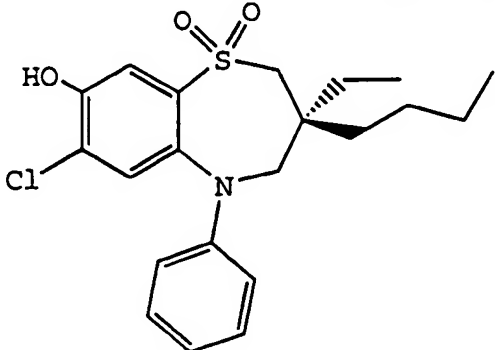
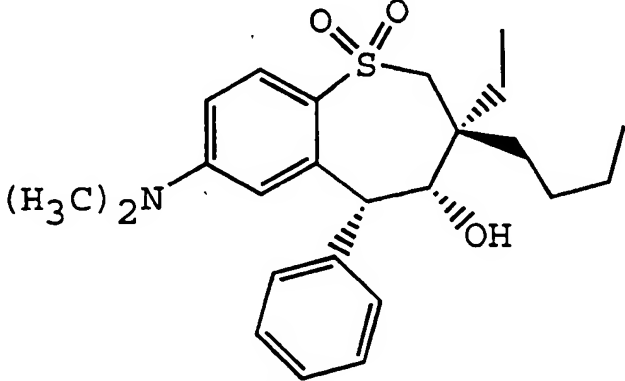
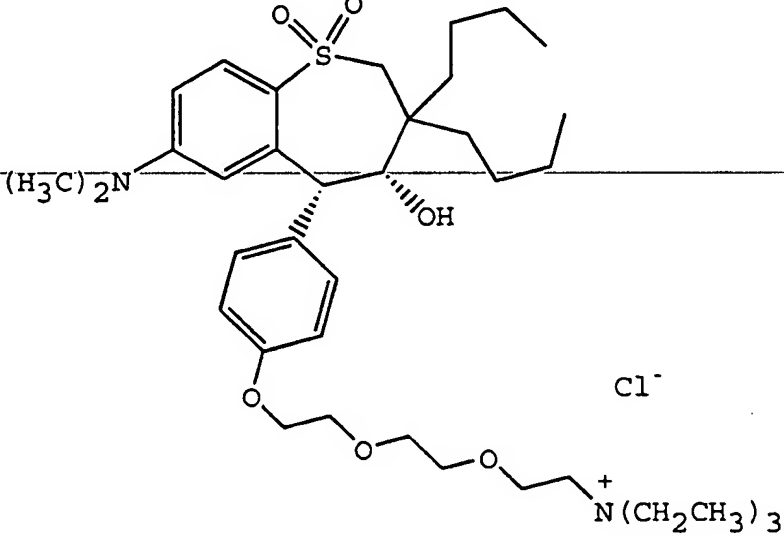
20 PCT/US97/04076, herein incorporated by reference. Still further IBAT inhibitors useful in the present invention are described in U.S. Application Serial No. 08/816,065, herein incorporated by reference. More IBAT inhibitor compounds useful in the present invention are described in  
25 WO 98/40375, herein incorporated by reference. Additional IBAT inhibitor compounds useful in the present invention are described in U.S. Application Serial No. 08/816,065, herein incorporated by reference. Further IBAT inhibiting compounds useful in the present invention are disclosed in  
30 U.S. Patent No. 5,994,391, herein incorporated by reference. IBAT inhibitors of particular interest in the present invention include those shown in Table 1, as well as the diastereomers, enantiomers, racemates, salts, and tautomers of the IBAT inhibitors of Table 1.

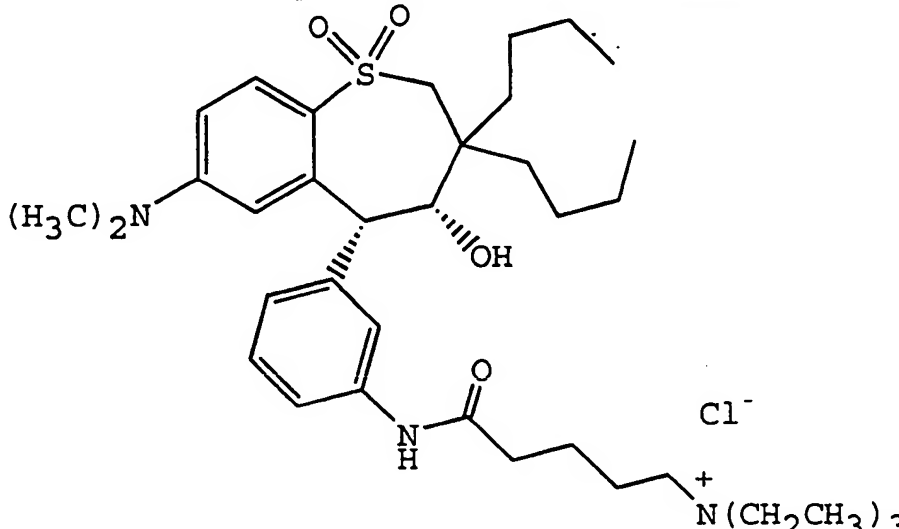
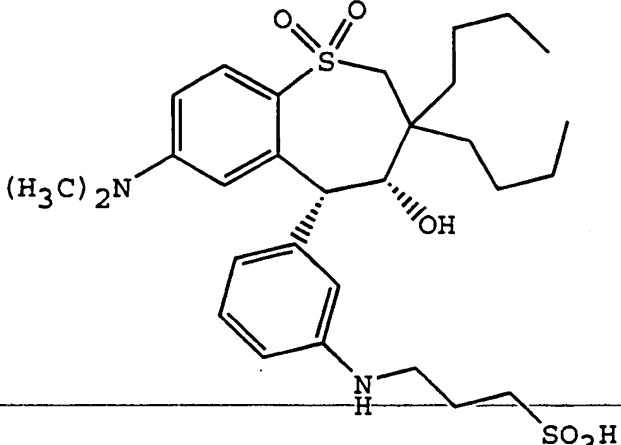
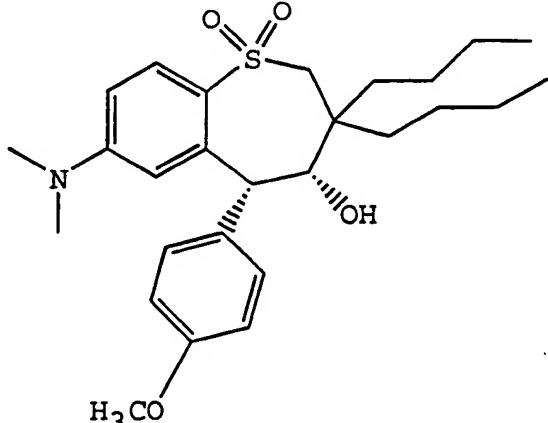
Table 1.

Compound Number	Structure
B-1	
B-2	 <p data-bbox="537 1262 1333 1346">(3R,5R) -3-butyl-3-ethyl-2,3,4,5-tetrahydro-7,8-dimethoxy-5-phenyl-1,4-benzothiazepine 1,1-dioxide</p>
B-3	

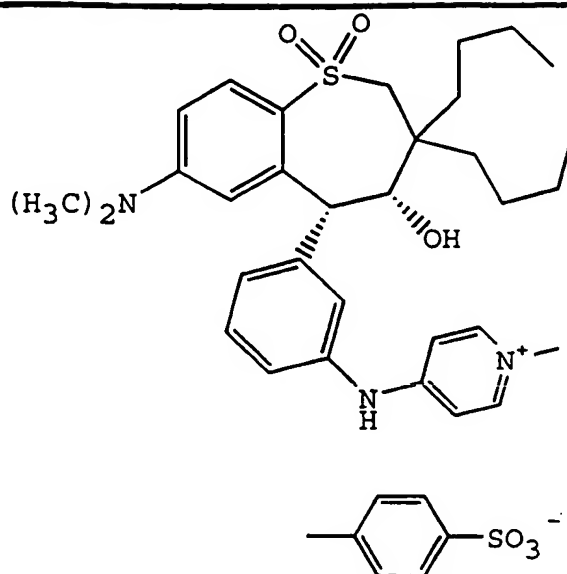
B-4	 <p>Chemical structure of compound B-4: A 1,4-dimethyl-2-phenyl-1,2,3,4-tetrahydro-2H-benzothiazine-6-sulfonate derivative. The structure features a central benzene ring fused to a six-membered ring containing a sulfonate group (SO<sub>2</sub>CH<sub>3</sub>) and a dimethylamino group (N(CH<sub>3</sub>)<sub>2</sub>). The six-membered ring is also substituted with a phenyl group and a hydroxyl group (OH). The phenyl group is further substituted with a 4-(4-(1,4-diazabicyclo[2.2.2]oct-7-yl)butoxy)benzyl group. The counterion is CH<sub>3</sub>SO<sub>3</sub><sup>-</sup>.</p>
B-5	 <p>Chemical structure of compound B-5: A 1,4-dimethyl-2-phenyl-1,2,3,4-tetrahydro-2H-benzothiazine-6-sulfonate derivative. The structure features a central benzene ring fused to a six-membered ring containing a sulfonate group (SO<sub>2</sub>CH<sub>3</sub>) and a dimethylamino group (N(CH<sub>3</sub>)<sub>2</sub>). The six-membered ring is also substituted with a phenyl group and a hydroxyl group (OH). The phenyl group is further substituted with a 4-(4-(1,4-diazabicyclo[2.2.2]oct-7-yl)butoxy)benzyl group. The counterion is CH<sub>3</sub>SO<sub>3</sub><sup>-</sup>.</p>
	 <p>Chemical structure of compound B-6: A 1,4-dimethyl-2-phenyl-1,2,3,4-tetrahydro-2H-benzothiazine-6-sulfonate derivative. The structure features a central benzene ring fused to a six-membered ring containing a sulfonate group (SO<sub>2</sub>CH<sub>3</sub>) and a dimethylamino group (N(CH<sub>3</sub>)<sub>2</sub>). The six-membered ring is also substituted with a phenyl group and a hydroxyl group (OH). The phenyl group is further substituted with a 4-(4-(1,4-diazabicyclo[2.2.2]oct-7-yl)butoxy)benzyl group. The counterion is Cl<sup>-</sup>.</p>
B-6	 <p>Chemical structure of compound B-6: A 1,4-dimethyl-2-phenyl-1,2,3,4-tetrahydro-2H-benzothiazine-6-sulfonate derivative. The structure features a central benzene ring fused to a six-membered ring containing a sulfonate group (SO<sub>2</sub>CH<sub>3</sub>) and a dimethylamino group (N(CH<sub>3</sub>)<sub>2</sub>). The six-membered ring is also substituted with a phenyl group and a hydroxyl group (OH). The phenyl group is further substituted with a 4-(4-(1,4-diazabicyclo[2.2.2]oct-7-yl)butoxy)benzyl group. The counterion is Cl<sup>-</sup>.</p>



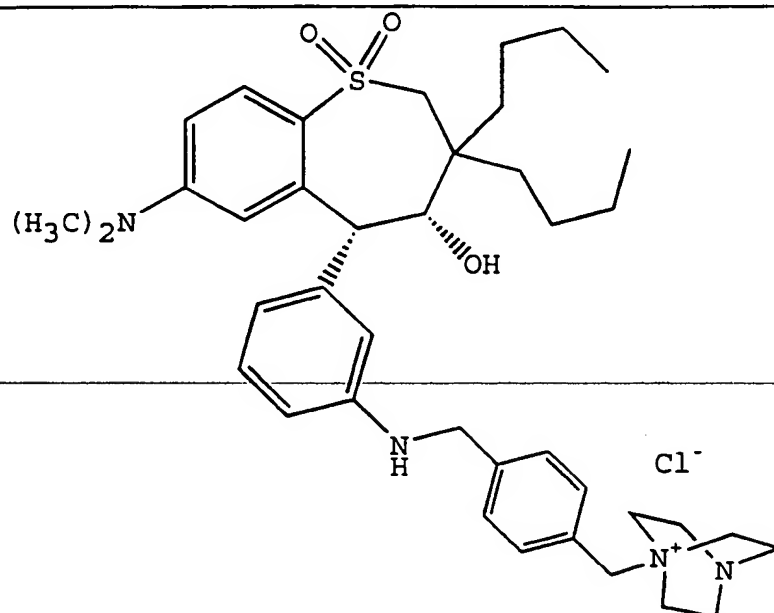
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B-8	
B-9	

B-10	 <p>Chemical structure of compound B-10: A 1,4-dihydro-2H-benzothiazepine derivative. The benzene ring has a dimethylamino group (<math>(\text{H}_3\text{C})_2\text{N}</math>) at the 6-position. The 3-position is substituted with a 4-((triethylammonium)butyl)benzamide group, shown with a dashed bond. The 4-position is substituted with a 1-hydroxy-1-phenylethyl group, shown with a wedged bond. The 7-position is substituted with a 1,1-dipropyl group. The sulfur atom is double-bonded to two oxygen atoms (<math>\text{S}(\text{O})_2</math>). The counterion is <math>\text{Cl}^-</math>.</p>
B-11	 <p>Chemical structure of compound B-11: A 1,4-dihydro-2H-benzothiazepine derivative. The benzene ring has a dimethylamino group (<math>(\text{H}_3\text{C})_2\text{N}</math>) at the 6-position. The 3-position is substituted with a 4-(sulfamoyl)benzamide group, shown with a dashed bond. The 4-position is substituted with a 1-hydroxy-1-phenylethyl group, shown with a wedged bond. The 7-position is substituted with a 1,1-dipropyl group. The sulfur atom is double-bonded to two oxygen atoms (<math>\text{S}(\text{O})_2</math>). The counterion is <math>\text{SO}_3\text{H}</math>.</p>
B-12	 <p>Chemical structure of compound B-12: A 1,4-dihydro-2H-benzothiazepine derivative. The benzene ring has a dimethylamino group (<math>\text{N}(\text{CH}_3)_2</math>) at the 6-position. The 3-position is substituted with a 4-(methoxy)phenyl group, shown with a dashed bond. The 4-position is substituted with a 1-hydroxy-1-phenylethyl group, shown with a wedged bond. The 7-position is substituted with a 1,1-dipropyl group. The sulfur atom is double-bonded to two oxygen atoms (<math>\text{S}(\text{O})_2</math>). The counterion is <math>\text{H}_3\text{CO}</math>.</p>

B-13

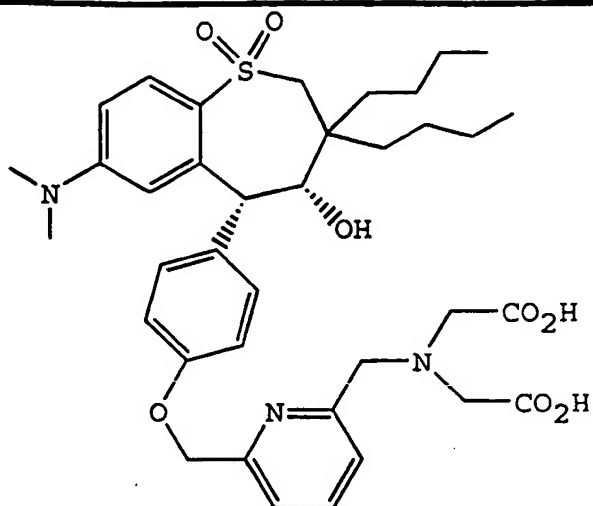


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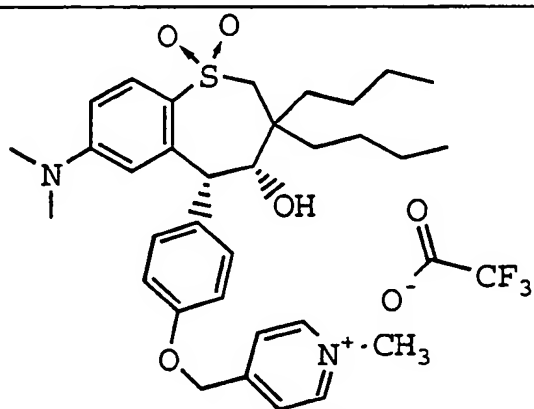




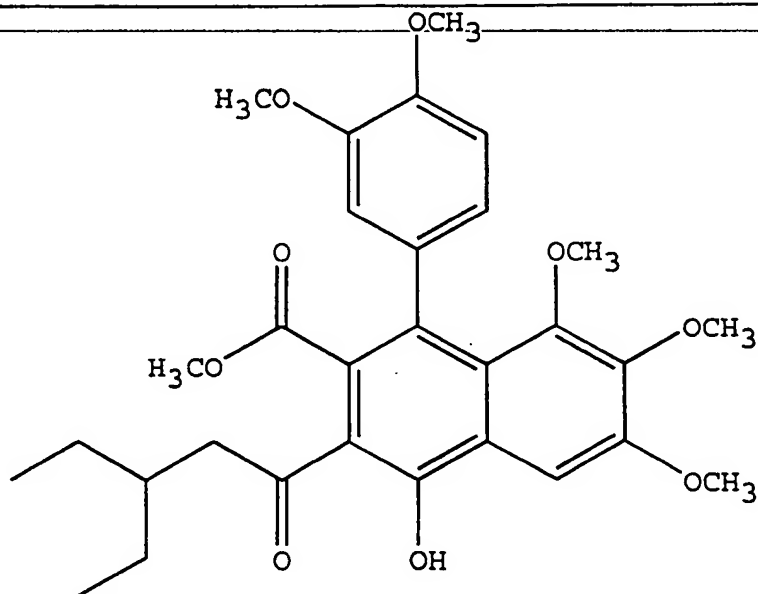
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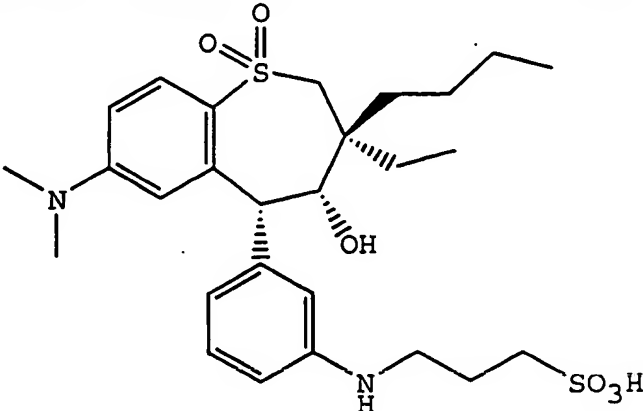
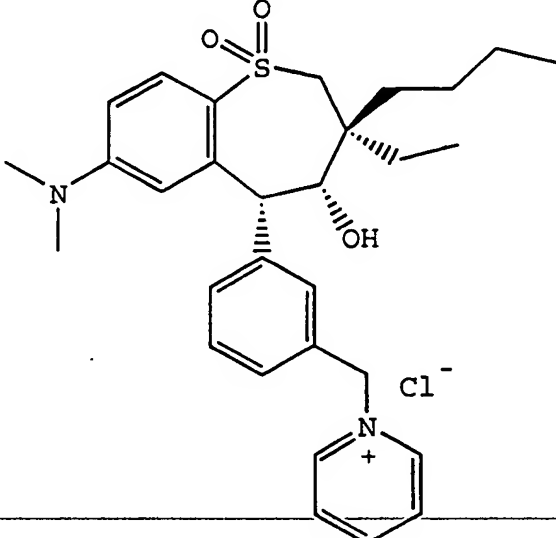
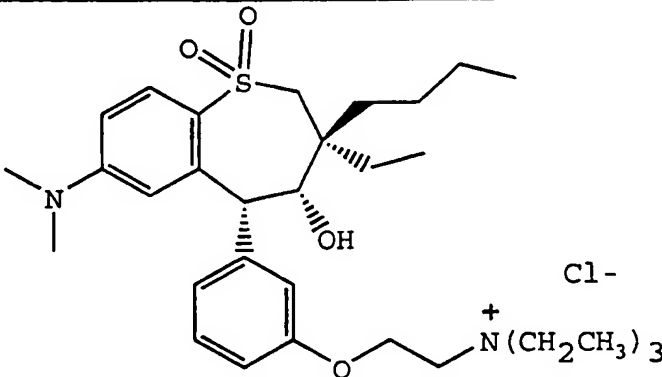
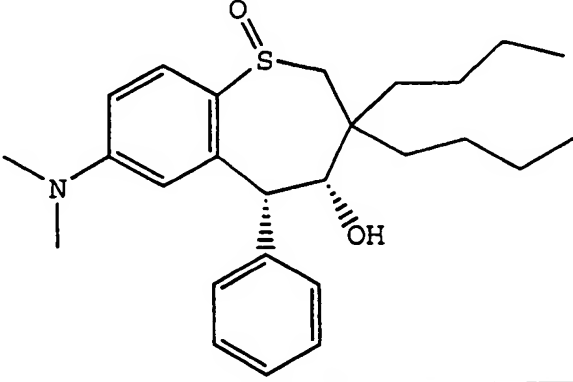
B-19



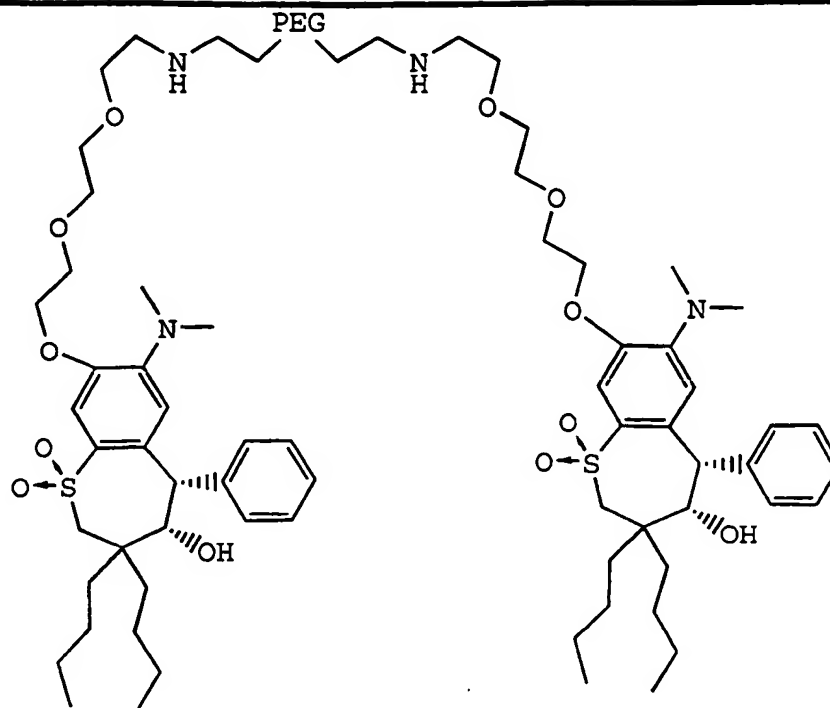
B-20



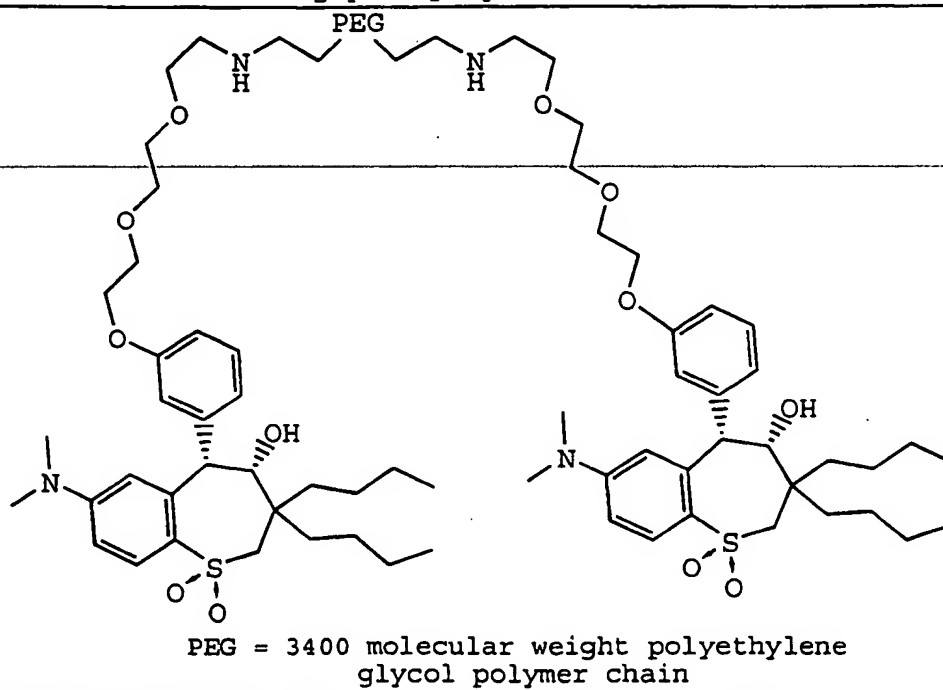


B-24	 <p>Chemical structure of compound B-24: A 1,4-dimethyl-5-phenyl-6-hydroxy-8-ethyl-8-methyl-8-oxo-8-phenyl-8-sulfonate derivative. The central ring is a 1,4-dimethyl-5-phenyl-6-hydroxy-8-ethyl-8-methyl-8-oxo-8-sulfonate derivative. The sulfonate group is attached to a phenyl ring via a methylene group. The phenyl ring is also substituted with a dimethylamino group and a hydroxyl group. The sulfonate group is shown as <math>\text{SO}_3\text{H}</math>.</p>
B-25	 <p>Chemical structure of compound B-25: A 1,4-dimethyl-5-phenyl-6-hydroxy-8-ethyl-8-methyl-8-oxo-8-sulfonate derivative. The central ring is a 1,4-dimethyl-5-phenyl-6-hydroxy-8-ethyl-8-methyl-8-oxo-8-sulfonate derivative. The sulfonate group is attached to a phenyl ring via a methylene group. The phenyl ring is also substituted with a dimethylamino group and a hydroxyl group. The sulfonate group is shown as <math>\text{Cl}^-</math>.</p>
B-26	 <p>Chemical structure of compound B-26: A 1,4-dimethyl-5-phenyl-6-hydroxy-8-ethyl-8-methyl-8-oxo-8-sulfonate derivative. The central ring is a 1,4-dimethyl-5-phenyl-6-hydroxy-8-ethyl-8-methyl-8-oxo-8-sulfonate derivative. The sulfonate group is attached to a phenyl ring via a methylene group. The phenyl ring is also substituted with a dimethylamino group and a hydroxyl group. The sulfonate group is shown as <math>\text{Cl}^-</math>.</p>
B-27	 <p>Chemical structure of compound B-27: A 1,4-dimethyl-5-phenyl-6-hydroxy-8-ethyl-8-methyl-8-oxo-8-sulfonate derivative. The central ring is a 1,4-dimethyl-5-phenyl-6-hydroxy-8-ethyl-8-methyl-8-oxo-8-sulfonate derivative. The sulfonate group is attached to a phenyl ring via a methylene group. The phenyl ring is also substituted with a dimethylamino group and a hydroxyl group. The sulfonate group is shown as <math>\text{N}^+(\text{CH}_2\text{CH}_3)_3</math>.</p>

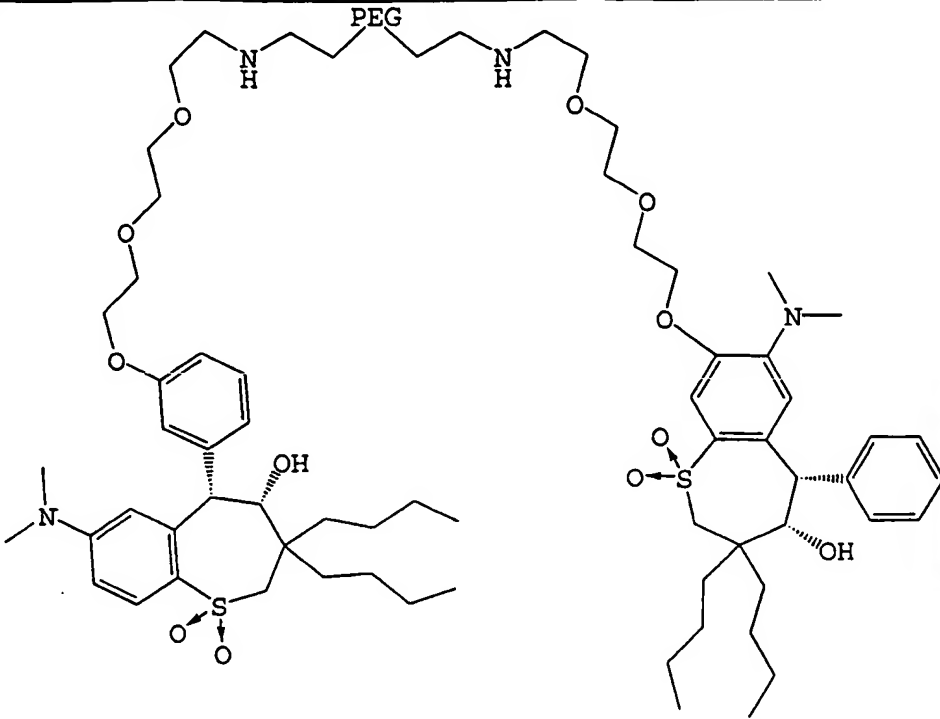
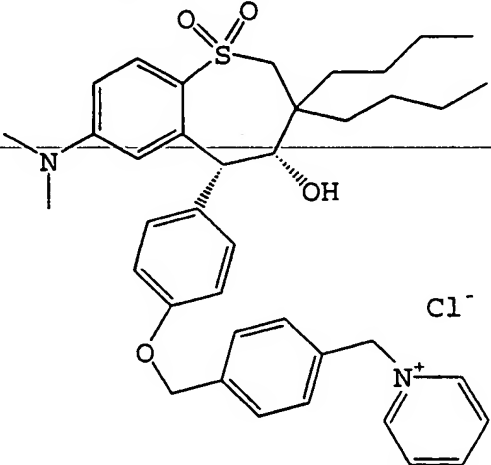
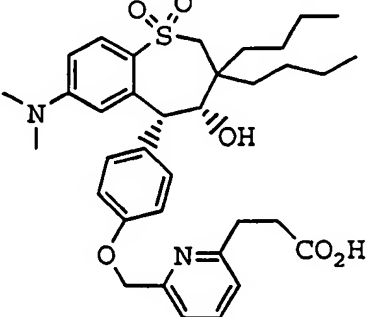
B-28

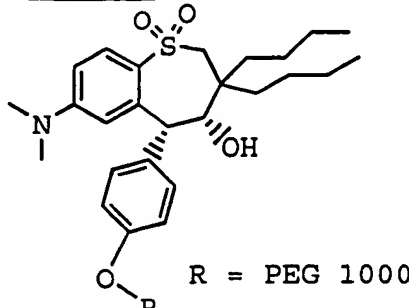
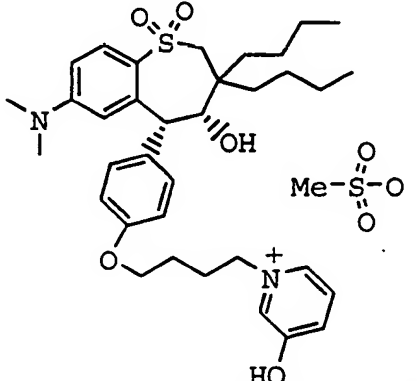
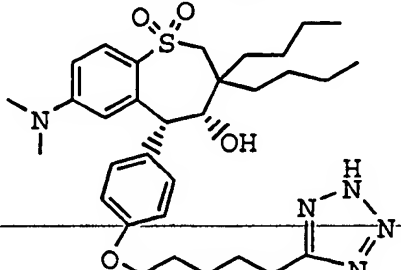
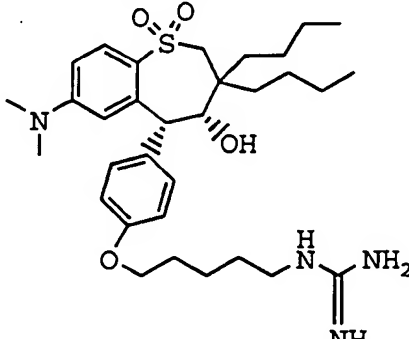
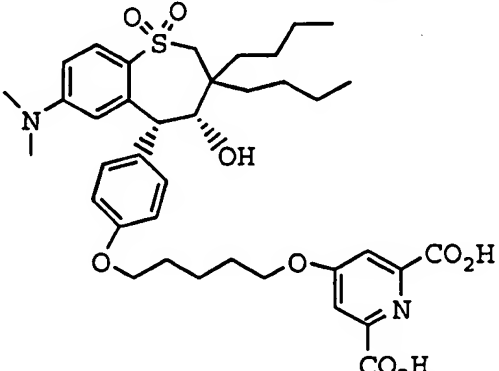


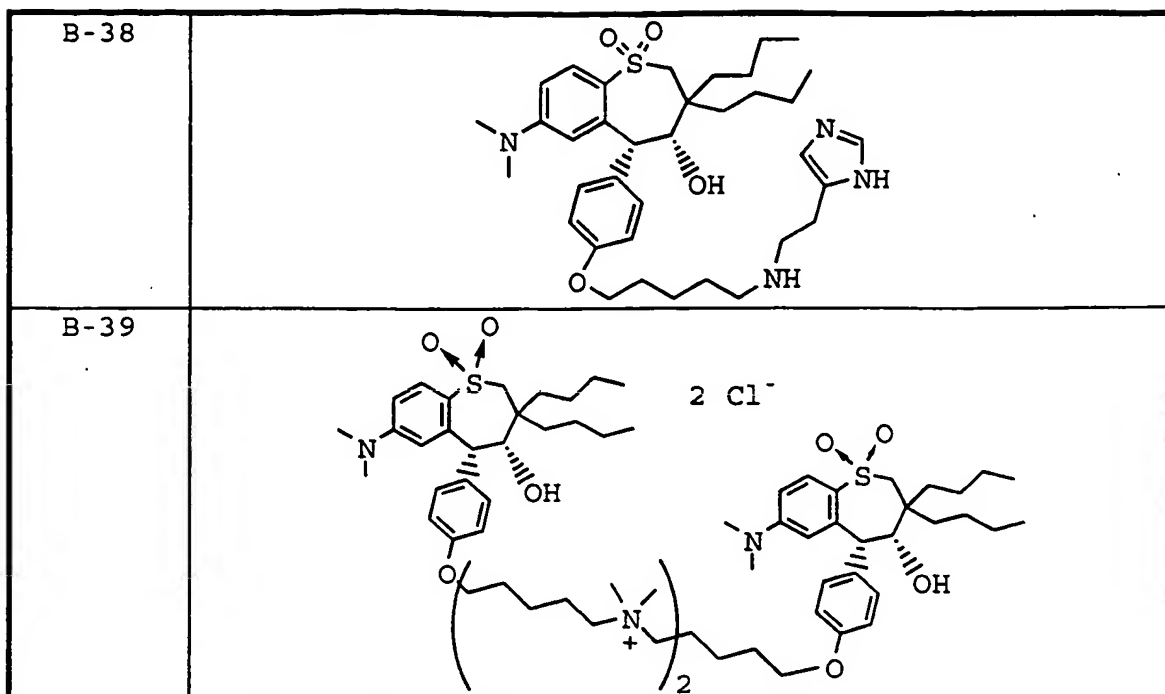
B-29





B-30	 <p>Chemical structure of compound B-30. It features a central 1,4-dithiane ring substituted with a 4-(dimethylamino)phenyl group, a 4-phenylphenyl group, a 4-phenylphenyl group, and a 4-phenylphenyl group. A long PEG chain (polyethylene glycol) is attached to the 4-phenylphenyl group. The PEG chain is labeled "PEG" and is connected to the 4-phenylphenyl group via an ether linkage. The PEG chain is also connected to the 4-phenylphenyl group via an ether linkage. The PEG chain is labeled "PEG" and is connected to the 4-phenylphenyl group via an ether linkage.</p> <p>PEG = 3400 molecular weight polyethylene glycol polymer chain</p>
B-31	 <p>Chemical structure of compound B-31. It features a central 1,4-dithiane ring substituted with a 4-(dimethylamino)phenyl group, a 4-phenylphenyl group, a 4-phenylphenyl group, and a 4-phenylphenyl group. A long PEG chain (polyethylene glycol) is attached to the 4-phenylphenyl group. The PEG chain is labeled "PEG" and is connected to the 4-phenylphenyl group via an ether linkage. The PEG chain is also connected to the 4-phenylphenyl group via an ether linkage. The PEG chain is labeled "PEG" and is connected to the 4-phenylphenyl group via an ether linkage.</p> <p>Cl<sup>-</sup></p>
B-32	 <p>Chemical structure of compound B-32. It features a central 1,4-dithiane ring substituted with a 4-(dimethylamino)phenyl group, a 4-phenylphenyl group, a 4-phenylphenyl group, and a 4-phenylphenyl group. A long PEG chain (polyethylene glycol) is attached to the 4-phenylphenyl group. The PEG chain is labeled "PEG" and is connected to the 4-phenylphenyl group via an ether linkage. The PEG chain is also connected to the 4-phenylphenyl group via an ether linkage. The PEG chain is labeled "PEG" and is connected to the 4-phenylphenyl group via an ether linkage.</p>

B-33	 <p>R = PEG 1000</p>
B-34	 <p>Me-SO<sub>3</sub><sup>-</sup></p>
B-35	
B-36	
B-37	



Fibric acid derivatives useful in the combinations and methods of the present invention comprise a wide variety of structures and functionalities. Preferred fibric acid derivative compounds for the present invention

5 are described in Table 2. The therapeutic compounds of Table 2 can be used in the present invention in a variety of forms, including acid form, salt form, racemates, enantiomers, zwitterions, and tautomers. The individual patent documents referenced in Table 2 are each herein

10 incorporated by reference.

Table 2.

Compound Number	Common Name	CAS Registry Number	Patent Document Reference
G-41	Clofibrate	637-07-0	U.S. 3,262,850
G-70	Fenofibrate	49562-28-9	U.S. 4,058,552
G-38	Ciprofibrate	52214-84-3	U.S. 3,948,973
G-20	Bezafibrate	41859-67-0	U.S. 3,781,328
G-78	Gemfibrozil	25182-30-1	U.S. 3,674,836
G-40	Clinofibrate	69047-39-8	U.S. 3,716,583
G-24	Binifibrate	30299-08-2	BE 884722

The compounds (for example, ileal bile acid transport inhibiting compounds or fibric acid derivative compounds) useful in the present invention can have no asymmetric carbon atoms, or, alternatively, the useful compounds can have one or more asymmetric carbon atoms. When the useful compounds have one or more asymmetric carbon atoms, they therefore include racemates and stereoisomers, such as diastereomers and enantiomers, in both pure form and in admixture. Such stereoisomers can be prepared using conventional techniques, either by reacting enantiomeric starting materials, or by separating isomers of compounds of the present invention.

Isomers may include geometric isomers, for example *cis*-isomers or *trans*-isomers across a double bond. All such isomers are contemplated among the compounds useful in the present invention.

The compounds useful in the present invention also include tautomers.

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The compounds useful in the present invention as discussed below include their salts, solvates and prodrugs.

#### Dosages, Formulations, and Routes of Administration

The compositions of the present invention can be administered for the prophylaxis and treatment of hyperlipidemic diseases or conditions by any means, preferably oral, that produce contact of these compounds with their site of action in the body, for example in the ileum, plasma, or liver of a mammal, e.g., a human.

For the prophylaxis or treatment of the conditions referred to above, the compounds useful in the compositions and methods of the present invention can be

used as the compound *per se*. Pharmaceutically acceptable salts are particularly suitable for medical applications because of their greater aqueous solubility relative to the parent compound. Such salts must clearly have a pharmaceutically acceptable anion or cation. Suitable pharmaceutically acceptable acid addition salts of the compounds of the present invention when possible include those derived from inorganic acids, such as hydrochloric, hydrobromic, phosphoric, metaphosphoric, nitric, sulfonic, and sulfuric acids, and organic acids such as acetic, benzenesulfonic, benzoic, citric, ethanesulfonic, fumaric, gluconic, glycolic, isothionic, lactic, lactobionic, maleic, malic, methanesulfonic, succinic, toluenesulfonic, tartaric, and trifluoroacetic acids. The chloride salt is particularly preferred for medical purposes. Suitable pharmaceutically acceptable base salts include ammonium salts, alkali metal salts such as sodium and potassium salts, and alkaline earth salts such as magnesium and calcium salts.

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The anions useful in the present invention are, of course, also required to be pharmaceutically acceptable and are also selected from the above list.

The compounds useful in the present invention can be presented with an acceptable carrier in the form of a pharmaceutical composition. The carrier must, of course, be acceptable in the sense of being compatible with the other ingredients of the composition and must not be deleterious to the recipient. The carrier can be a solid or a liquid, or both, and is preferably formulated with the compound as a unit-dose composition, for example, a tablet, which can contain from 0.05% to 95% by weight of the active compound. Other pharmacologically active substances can also be present, including other compounds of the present invention. The pharmaceutical compositions

of the invention can be prepared by any of the well known techniques of pharmacy, consisting essentially of admixing the components.

Optionally, the combination of the present invention  
5 can comprise a composition comprising an ileal bile acid transport inhibiting compound and a fibric acid derivative. In such a composition, the ileal bile acid transport inhibiting compound and the bile acid sequestering compound can be present in a single dosage  
10 form, for example a pill, a capsule, or a liquid which contains both of the compounds.

These compounds can be administered by any conventional means available for use in conjunction with pharmaceuticals, either as individual therapeutic  
15 compounds or as a combination of therapeutic compounds.

The amount of compound which is required to achieve the desired biological effect will, of course, depend on a number of factors such as the specific compound chosen, the use for which it is intended, the mode of  

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20 administration, and the clinical condition of the recipient.

In general, a total daily dose of an IBAT inhibitor can be in the range of from about 0.01 to about 1000 mg/day, preferably from about 0.1 mg to about 50 mg/day,  
25 more preferably from about 1 to about 10 mg/day.

A total daily dose of a fibric acid derivative can generally be in the range of from about 1000 to about 3000 mg/day in single or divided doses. Gemfibrozil or  
30 clinofibrate, for example, are frequently each administered separately in a 1200 mg/day dose. Clofibrate is frequently administered in a 2000 mg/day dose. Binifibrate is frequently administered in a 1800 mg/day dose.

The daily doses described in the preceding paragraphs for the various therapeutic compounds can be administered to the patient in a single dose, or in proportionate multiple subdoses. Subdoses can be administered 2 to 6  
5 times per day. Doses can be in sustained release form effective to obtain desired results.

In the case of pharmaceutically acceptable salts, the weights indicated above refer to the weight of the acid equivalent or the base equivalent of the therapeutic  
10 compound derived from the salt.

Oral delivery of the combinations of the present invention can include formulations, as are well known in the art, to provide prolonged or sustained delivery of the drug to the gastrointestinal tract by any number of  
15 mechanisms. These include, but are not limited to, pH sensitive release from the dosage form based on the changing pH of the small intestine, slow erosion of a tablet or capsule, retention in the stomach based on the ~~physical properties of the formulation, bioadhesion of the~~  
20 dosage form to the mucosal lining of the intestinal tract, or enzymatic release of the active drug from the dosage form. For some of the therapeutic compounds useful in the present invention (e.g., an IBAT inhibitor or a fibric acid derivative), the intended effect is to extend the  
25 time period over which the active drug molecule is delivered to the site of action (e.g., the ileum) by manipulation of the dosage form. Thus, enteric-coated and enteric-coated controlled release formulations are within the scope of the present invention. Suitable enteric  
30 coatings include cellulose acetate phthalate, polyvinylacetate phthalate, hydroxypropylmethylcellulose phthalate and anionic polymers of methacrylic acid and methacrylic acid methyl ester.

The combinations of the present invention can be delivered orally either in a solid, in a semi-solid, or in a liquid form. When in a liquid or in a semi-solid form, the combinations of the present invention can, for  
5 example, be in the form of a liquid, syrup, or contained in a gel capsule (e.g., a gel cap).

Pharmaceutical compositions according to the present invention include those suitable for oral, rectal, topical, buccal (e.g., sublingual), and parenteral (e.g.,  
10 subcutaneous, intramuscular, intradermal, or intravenous) administration, although the most suitable route in any given case will depend on the nature and severity of the condition being treated and on the nature of the particular compound which is being used. In most cases,  
15 the preferred route of administration is oral.

Pharmaceutical compositions suitable for oral administration can be presented in discrete units, such as capsules, cachets, lozenges, or tablets, each containing a  
predetermined amount of at least one therapeutic compound  
20 useful in the present invention; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water or water-in-oil emulsion. As indicated, such compositions can be prepared by any suitable method of pharmacy which includes the step of  
25 bringing into association the active compound(s) and the carrier (which can constitute one or more accessory ingredients). In general, the compositions are prepared by uniformly and intimately admixing the active compound with a liquid or finely divided solid carrier, or both,  
30 and then, if necessary, shaping the product. For example, a tablet can be prepared by compressing or molding a powder or granules of the compound, optionally with one or more accessory ingredients. Compressed tablets can be prepared by compressing, in a suitable machine, the



compound in a free-flowing form, such as a powder or granules optionally mixed with a binder, lubricant, inert diluent and/or surface active/dispersing agent(s). Molded tablets can be made by molding, in a suitable machine, the powdered compound moistened with an inert liquid diluent.

Pharmaceutical compositions suitable for buccal (sub-lingual) administration include lozenges comprising a compound of the present invention in a flavored base, usually sucrose, and acacia or tragacanth, and pastilles comprising the compound in an inert base such as gelatin and glycerin or sucrose and acacia.

Pharmaceutical compositions suitable for parenteral administration conveniently comprise sterile aqueous preparations of a compound of the present invention. These preparations are preferably administered intravenously, although administration can also be effected by means of subcutaneous, intramuscular, or intradermal injection. Such preparations can conveniently be prepared by admixing the compound with water and rendering the resulting solution sterile and isotonic with the blood. Injectable compositions according to the invention will generally contain from 0.1 to 5% w/w of a compound disclosed herein.

Pharmaceutical compositions suitable for rectal administration are preferably presented as unit-dose suppositories. These can be prepared by admixing a compound of the present invention with one or more conventional solid carriers, for example, cocoa butter, and then shaping the resulting mixture.

Pharmaceutical compositions suitable for topical application to the skin preferably take the form of an ointment, cream, lotion, paste, gel, spray, aerosol, or oil. Carriers which can be used include petroleum jelly (e.g., Vaseline), lanolin, polyethylene glycols, alcohols, and combinations of two or more thereof. The active

compound is generally present at a concentration of from 0.1 to 50% w/w of the composition, for example, from 0.5 to 2%.

Transdermal administration is also possible.

- 5 Pharmaceutical compositions suitable for transdermal administration can be presented as discrete patches adapted to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. Such patches suitably contain a compound of the present
- 10 invention in an optionally buffered, aqueous solution, dissolved and/or dispersed in an adhesive, or dispersed in a polymer. A suitable concentration of the active compound is about 1% to 35%, preferably about 3% to 15%. As one particular possibility, the compound can be
- 15 delivered from the patch by electrotransport or iontophoresis, for example, as described in Pharmaceutical Research, 3(6), 318 (1986).

In any case, the amount of active ingredient that can be combined with carrier materials to produce a single

- 
- 20 dosage form to be administered will vary depending upon the host treated and the particular mode of administration.

- The solid dosage forms for oral administration including capsules, tablets, pills, powders, gel caps, and
- 25 granules noted above comprise one or more compounds useful in the present invention admixed with at least one inert diluent such as sucrose, lactose, or starch. Such dosage forms may also comprise, as in normal practice, additional substances other than inert diluents, e.g., lubricating
- 30 agents such as magnesium stearate or solubilizing agents such as cyclodextrins. In the case of capsules, tablets, powders, granules, gel caps, and pills, the dosage forms may also comprise buffering agents. Tablets and pills can additionally be prepared with enteric coatings.

Liquid dosage forms for oral administration can include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs containing inert diluents commonly used in the art, such as water. Such compositions may also comprise adjuvants, such as wetting agents, emulsifying and suspending agents, and sweetening, flavoring, and perfuming agents.

Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing or setting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water,

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Ringer's solution, and isotonic sodium chloride solution.

In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this

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purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

Pharmaceutically acceptable carriers encompass all the foregoing and the like.

In combination therapy, administration of two or more of the therapeutic agents useful in the present invention may take place sequentially in separate formulations, or may be accomplished by simultaneous administration in a single formulation or separate formulations.

Administration may be accomplished by oral route, or by intravenous, intramuscular, or subcutaneous injections. The formulation may be in the form of a bolus, or in the form of aqueous or non-aqueous isotonic sterile injection

solutions or suspensions. These solutions and suspensions may be prepared from sterile powders or granules having one or more pharmaceutically-acceptable carriers or diluents, or a binder such as gelatin or

- 5 hydroxypropylmethyl cellulose, together with one or more of a lubricant, preservative, surface active or dispersing agent.

For oral administration, the pharmaceutical composition may be in the form of, for example, a tablet,  
10 capsule, suspension, or liquid. Capsules, tablets, etc., can be prepared by conventional methods well known in the art. The pharmaceutical composition is preferably made in the form of a dosage unit containing a particular amount of the active ingredient or ingredients. Examples of  
15 dosage units are tablets or capsules. These may with advantage contain one or more therapeutic compound in an amount described above. For example, in the case of an IBAT inhibitor, the dose range may be from about 0.01  
~~mg/day to about 500 mg/day or any other dose, dependent~~

- 20 upon the specific inhibitor, as is known in the art. Also by way of example, in the case of a fibric acid derivative, the dose range may be from about 0.01 mg to about 500 mg or any other dose, dependent upon the specific inhibitor, as is known in the art.

- 25 The active ingredients may also be administered by injection as a composition wherein, for example, saline, dextrose, or water may be used as a suitable carrier. A suitable daily dose of each active therapeutic compound is one that achieves the same blood serum level as produced  
30 by oral administration as described above.

The therapeutic compounds may further be administered by any combination of oral/oral, oral/parenteral, or parenteral/parenteral route.

Pharmaceutical compositions for use in the treatment methods of the present invention may be administered in oral form or by intravenous administration. Oral administration of the combination therapy is preferred.

- 5 Dosing for oral administration may be with a regimen calling for single daily dose, or for a single dose every other day, or for multiple, spaced doses throughout the day. The therapeutic compounds which make up the combination therapy may be administered simultaneously, 10 either in a combined dosage form or in separate dosage forms intended for substantially simultaneous oral administration. The therapeutic compounds which make up the combination therapy may also be administered sequentially, with either therapeutic compound being 15 administered by a regimen calling for two-step ingestion. Thus, a regimen may call for sequential administration of the therapeutic compounds with spaced-apart ingestion of the separate, active agents. The time period between the multiple ingestion steps may range from a few minutes to 20 several hours, depending upon the properties of each therapeutic compound such as potency, solubility, bioavailability, plasma half-life and kinetic profile of the therapeutic compound, as well as depending upon the effect of food ingestion and the age and condition of the 25 patient. Circadian variation of the target molecule concentration may also determine the optimal dose interval. The therapeutic compounds of the combined therapy whether administered simultaneously, substantially simultaneously, or sequentially, may involve a regimen 30 calling for administration of one therapeutic compound by oral route and another therapeutic compound by intravenous route. Whether the therapeutic compounds of the combined therapy are administered by oral or intravenous route, separately or together, each such therapeutic compound

will be contained in a suitable pharmaceutical formulation of pharmaceutically-acceptable excipients, diluents or other formulations components. Examples of suitable pharmaceutically-acceptable formulations containing the  
5 therapeutic compounds for oral administration are given above.

#### Treatment Regimen

The dosage regimen to prevent, give relief from, or  
10 ameliorate a disease condition having hyperlipemia as an element of the disease, e.g., atherosclerosis, or to protect against or treat further high cholesterol plasma or blood levels with the compounds and/or compositions of the present invention is selected in accordance with a  
15 variety of factors. These include the type, age, weight, sex, diet, and medical condition of the patient, the severity of the disease, the route of administration, pharmacological considerations such as the activity,  
~~efficacy, pharmacokinetics and toxicology profiles of the~~  
20 particular compound employed, whether a drug delivery system is utilized, and whether the compound is administered as part of a drug combination. Thus, the dosage regimen actually employed may vary widely and therefore deviate from the preferred dosage regimen set  
25 forth above.

Initial treatment of a patient suffering from a hyperlipidemic condition can begin with the dosages indicated above. Treatment should generally be continued as necessary over a period of several weeks to several  
30 months or years until the hyperlipidemic disease condition has been controlled or eliminated. Patients undergoing treatment with the compounds or compositions disclosed herein can be routinely monitored by, for example, measuring serum LDL and total cholesterol levels by any of

the methods well known in the art, to determine the effectiveness of the combination therapy. Continuous analysis of such data permits modification of the treatment regimen during therapy so that optimal effective  
5 amounts of each type of therapeutic compound are administered at any point in time, and so that the duration of treatment can be determined as well. In this way, the treatment regimen/dosing schedule can be rationally modified over the course of therapy so that the  
10 lowest amount of the therapeutic compounds which together exhibit satisfactory effectiveness is administered, and so that administration is continued only so long as is necessary to successfully treat the hyperlipidemic condition.

15 A potential advantage of the combination therapy disclosed herein may be reduced dosage amount of any individual therapeutic compound, or all therapeutic compounds, effective in treating hyperlipidemic conditions  
~~such as atherosclerosis and hypercholesterolemia. The~~  
20 dosage lowering will provide advantages including reduction of side effects of the individual therapeutic compounds when compared to the monotherapy.

One of the several embodiments of the present invention comprises a combination therapy comprising the  
25 use of a first amount of an IBAT inhibitor and a second amount of another cardiovascular therapeutic useful in the prophylaxis or treatment of hyperlipidemia or atherosclerosis, wherein said first and second amounts together comprise an anti-hyperlipidemic condition  
30 effective amount or an anti-atherosclerotic condition effective amount of said compounds. For example one of the many embodiments of the present invention is a combination therapy comprising therapeutic dosages of an IBAT inhibitor and a fibric acid derivative. A preferred

embodiment of the present invention is a combination therapy comprising therapeutic dosages of a benzothiepine IBAT inhibitor and a fibric acid derivative.

The following non-limiting examples serve to  
5 illustrate various aspects of the present invention.

c. Examples

Table 3 illustrates examples of some combinations of  
10 the present invention wherein the combination comprises a first amount of an IBAT inhibitor and a second amount of a fibric acid derivative, wherein said first and second amounts together comprise an anti-hyperlipidemic condition effective amount or an anti-atherosclerotic condition  
15 effective amount of said compounds.

Table 3.

Example Number	Component 1	Component 2
1	B-1	clofibrate
2	B-2	clofibrate
3	B-3	clofibrate
4	B-4	clofibrate
5	B-5	clofibrate
6	B-6	clofibrate
7	B-7	clofibrate
8	B-8	clofibrate
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19	B-19	clofibrate



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35	B-35	clofibrate
36	B-36	clofibrate
37	B-37	clofibrate
38	B-38	clofibrate
39	B-39	clofibrate
40	B-1	fenofibrate
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42	B-3	fenofibrate
43	B-4	fenofibrate
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49	B-10	fenofibrate
50	B-11	fenofibrate
51	B-12	fenofibrate
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69	B-30	fenofibrate
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75	B-36	fenofibrate
76	B-37	fenofibrate
77	B-38	fenofibrate
78	B-39	fenofibrate
79	B-1	ciprofibrate
80	B-2	ciprofibrate
81	B-3	ciprofibrate
82	B-4	ciprofibrate
83	B-5	ciprofibrate
84	B-6	ciprofibrate
85	B-7	ciprofibrate
86	B-8	ciprofibrate
87	B-9	ciprofibrate
88	B-10	ciprofibrate
89	B-11	ciprofibrate
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110	B-32	ciprofibrate
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154	B-37	bezafibrate
155	B-38	bezafibrate
156	B-39	bezafibrate
157	B-1	gemfibrozil
158	B-2	gemfibrozil
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163	B-7	gemfibrozil
164	B-8	gemfibrozil
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166	B-10	gemfibrozil
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192	B-36	gemfibrozil
193	B-37	gemfibrozil
194	B-38	gemfibrozil
195	B-39	gemfibrozil
196	B-1	clinfibrate
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233	B-38	clinofibrate
234	B-39	clinofibrate
235	B-1	binifibrate
236	B-2	binifibrate
237	B-3	binifibrate
238	B-4	binifibrate
239	B-5	binifibrate
240	B-6	binifibrate
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272	B-38	binifibrate
273	B-39	binifibrate

### BIOLOGICAL ASSAYS

The utility of the combinations of the present invention can be shown by the following assays. These assays are performed *in vitro* and in animal models essentially using procedures recognized to show the utility of the present invention.

#### 10 In Vitro Assay of compounds that inhibit IBAT-mediated uptake of [<sup>14</sup>C]-Taurocholate (TC) in H14 Cells

Baby hamster kidney cells (BHK) transfected with the cDNA of human IBAT (H14 cells) are to be seeded at 60,000 cells/well in 96 well Top-Count tissue culture plates for assays run within in 24 hours of seeding, 30,000 cells/well for assays run within 48 hours, and 10,000 cells/well for assays run within 72 hours.

On the day of assay, the cell monolayer is gently washed once with 100 µl assay buffer (Dulbecco's Modified Eagle's medium with 4.5 g/L glucose + 0.2% (w/v) fatty acid free bovine serum albumin- (FAF)BSA). To each well 50 µl of a two-fold concentrate of test compound in assay buffer is added along with 50 µl of 6 µM [<sup>14</sup>C]-taurocholate in assay buffer (final concentration of 3 µM [<sup>14</sup>C]-taurocholate). The cell culture plates are incubated 2 hours at 37°C prior to gently washing each well twice with 100 µl 4°C Dulbecco's phosphate-buffered saline (PBS)

containing 0.2% (w/v) (FAF)BSA. The wells are then to be gently washed once with 100  $\mu$ l 4°C PBS without (FAF)BSA. To each 200  $\mu$ l of liquid scintillation counting fluid is to be added, the plates are heat sealed and shaken for 30 minutes at room temperature prior to measuring the amount of radioactivity in each well on a Packard Top-Count instrument.

In Vitro Assay of compounds that inhibit uptake of [ $^{14}$ C]-

10 Alanine

The alanine uptake assay can be performed in an identical fashion to the taurocholate assay, with the exception that labeled alanine is to be substituted for the labeled taurocholate.

15

Measurement of Rat Fecal Bile Acid Concentration (FBA)

Total fecal output from individually housed rats is to be collected for 24 or 48 hours, dried under a stream of nitrogen, pulverized, mixed, and weighed. Approximately 0.1 gram is weighed out and extracted into an organic solvent (butanol/water). Following separation and drying, the residue is dissolved in methanol and the amount of bile acid present will be measured enzymatically using the 3 $\alpha$ -hydroxysteroid steroid dehydrogenase reaction with bile acids to reduce NAD. (see Mashige, F. et al. Clin. Chem., 27, 1352 (1981), herein incorporated by reference).

Rat Gavage Assay

Male Wister rats (275-300g) are to be administered IBAT inhibitors using an oral gavage procedure. Drug or vehicle (0.2% TWEEN 80 in water) is administered once a day (9:00-10:0 a.m.) for 4 days at varying dosages in a final volume of 2 mL per kilogram of body weight. (TWEEN



80 is a 20 molar polyethyleneoxide sorbitan monooleate surfactant manufactured by ICI Specialty Chemicals, Wilmington, Delaware, U.S.A.) Total fecal samples are collected during the final 48 hours of the treatment period and analyzed for bile acid content using an enzymatic assay as described below. Compound efficacy will be determined by comparison of the increase in fecal bile acid (FBA) concentration in treated rats to the mean FBA concentration of rats in the vehicle group.

10

[<sup>3</sup>H]Taurocholate Uptake in Rabbit Brush Border Membrane Vesicles (BBMV)

Rabbit ileal brush border membranes are to be prepared from frozen ileal mucosa by the calcium precipitation method describe by Malathi et al. (Biochimica Biophysica Acta, 554, 259 (1979), herein incorporated by reference). The method for measuring taurocholate is essentially as described by Kramer et al. (Biochimica Biophysica Acta, 1111, 93 (1992), herein incorporated by reference) except the assay volume will be 200  $\mu$ l instead of 100  $\mu$ l. Briefly, at room temperature a 190  $\mu$ l solution containing 2 $\mu$ M [<sup>3</sup>H]-taurocholate(0.75  $\mu$ Ci), 20 mM tris, 100 mM NaCl, 100 mM mannitol pH 7.4 is incubated for 5 sec with 10  $\mu$ l of brush border membrane vesicles (60-120  $\mu$ g protein). The incubation is initiated by the addition of the BBMV while vortexing and the reaction is to be stopped by the addition of 5 ml of ice cold buffer (20 mM Hepes-tris, 150 mM KCl) followed immediately by filtration through a nylon filter (0.2  $\mu$ m pore) and an additional 5 ml wash with stop buffer.

30

Acyl-CoA; Cholesterol Acyl Transferase (ACAT)

Hamster liver and rat intestinal microsomes are to be prepared from tissue as described previously (J. Biol.

Chem., 255, 9098 (1980), herein incorporated by reference) and used as a source of ACAT enzyme. The assay will consist of a 2.0 ml incubation containing 24  $\mu$ M Oleoyl-CoA (0.05  $\mu$ Ci) in a 50 mM sodium phosphate, 2 mM DTT pH 7.4  
5 buffer containing 0.25 % BSA and 200  $\mu$ g of microsomal protein. The assay will be initiated by the addition of oleoyl-CoA. The reaction proceeds for 5 min at 37° C and will be terminated by the addition of 8.0 ml of chloroform/ methanol (2:1). To the extraction is added  
10 125  $\mu$ g of cholesterol oleate in chloroform methanol to act as a carrier and the organic and aqueous phases of the extraction are separated by centrifugation after thorough vortexing. The chloroform phase is to be taken to dryness and then spotted on a silica gel 60 TLC plate and  
15 developed in hexane/ethyl ether (9:1). The amount of cholesterol ester formed will be determined by measuring the amount of radioactivity incorporated into the cholesterol oleate spot on the TLC plate with a Packard Instaimager.

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Measurement of Hepatic Cholesterol Concentration (HEPATIC CHOL)

Liver tissue is to be weighed and homogenized in chloroform:methanol (2:1). After homogenization and  
25 centrifugation the supernatant is separated and dried under nitrogen. The residue is to be dissolved in isopropanol and the cholesterol content will be measured enzymatically, using a combination of cholesterol oxidase and peroxidase, as described by Allain, C. A. et al.,  
30 Clin. Chem., 20, 470 (1974) (herein incorporated by reference).

Measurement of Hepatic HMG CoA-Reductase Activity (HMG COA)

Hepatic microsomes are to be prepared by homogenizing liver samples in a phosphate/sucrose buffer, followed by centrifugal separation. The final pelleted material is resuspended in buffer and an aliquot will be assayed for HMG CoA reductase activity by incubating for 60 minutes at 37° C in the presence of <sup>14</sup>C-HMG-CoA (Dupont-NEN). The reaction is stopped by adding 6N HCl followed by centrifugation. An aliquot of the supernatant is separated, by thin-layer chromatography, and the spot corresponding to the enzyme product is scraped off the plate, extracted and radioactivity is determined by scintillation counting. (Reference: Akerlund, J. and Bjorkhem, I. (1990) *J. Lipid Res.* 31, 2159).

15 Measurement of Hepatic Cholesterol 7- $\alpha$ -Hydroxylase Activity (7 $\alpha$ -OHase)

Hepatic microsomes are to be prepared by homogenizing liver samples in a phosphate/sucrose buffer, followed by centrifugal separation. The final pelleted material is resuspended in buffer and an aliquot will be assayed for cholesterol 7- $\alpha$ -hydroxylase activity by incubating for 5 minutes at 37° C in the presence of NADPH. Following extraction into petroleum ether, the organic solvent is evaporated and the residue is dissolved in acetonitrile/methanol. The enzymatic product will be separated by injecting an aliquot of the extract onto a C<sub>18</sub> reversed phase HPLC column and quantitating the eluted material using UV detection at 240nm. (Reference: Horton, J. D., et al. (1994) *J. Clin. Invest.* 93, 2084).

30

Determination of Serum Cholesterol (SER.CHOL, HDL-CHOL, TGI and VLDL + LDL)

Total serum cholesterol (SER.CHOL) are to be measured enzymatically using a commercial kit from Wako Fine Chemicals (Richmond, VA); Cholesterol C11, Catalog No. 276-64909. HDL cholesterol (HDL-CHOL) will be assayed using this same kit after precipitation of VLDL and LDL with Sigma Chemical Co. HDL Cholesterol reagent, Catalog No. 352-3 (dextran sulfate method). Total serum triglycerides (blanked) (TGI) will be assayed enzymatically with Sigma Chemical Co. GPO-Trinder, Catalog No. 337-B. VLDL and LDL (VLDL + LDL) cholesterol concentrations will be calculated as the difference between total and HDL cholesterol.

#### Measurement of Hamster Fecal Bile Acid Concentration (FBA)

Total fecal output from individually housed hamsters is to be collected for 24 or 48 hours, dried under a stream of nitrogen, pulverized and weighed. Approximately 0.1 gram is weighed out and extracted into an organic solvent (butanol/water). Following separation and drying, the residue is dissolved in methanol and the amount of bile acid present is measured enzymatically using the 3 $\alpha$ -hydroxysteroid steroid dehydrogenase reaction with bile acids to reduce NAD. (Mashige, F. et al. Clin. Chem., 27, 1352 (1981), herein incorporated by reference).

#### Dog Model for Evaluating Lipid Lowering Drugs

Male beagle dogs, obtained from a vendor such as Marshall farms and weighing 6-12 kg are fed once a day for two hours and given water ad libitum. Dogs may be randomly assigned to a dosing groups consisting of 6 to 12 dogs each, such as: vehicle, i.g.; 1mg/kg, i.g.; 2mg/kg, i.g.; 4 mg/kg, i.g.; 2 mg/kg, p.o. (powder in capsule). Intra-gastric dosing of a therapeutic material dissolved in aqueous solution (for example, 0.2% Tween 80 solution

[polyoxyethylene mono-oleate, Sigma Chemical Co., St. Louis, MO]) may be done using a gavage tube. Prior to initiating dosing, blood samples may be drawn from the cephalic vein in the morning before feeding in order to  
5 evaluate serum cholesterol (total and HDL) and triglycerides. For several consecutive days animals are dosed in the morning, prior to feeding. Animals are to be allowed 2 hours to eat before any remaining food is removed. Feces are to be collected over a 2 day period at  
10 the end of the study and may be analyzed for bile acid or lipid content. Blood samples are also to be taken, at the end of the treatment period, for comparison with pre-study serum lipid levels. Statistical significance will be determined using the standard student's T-test with  $p < .05$ .

15

#### Dog Serum Lipid Measurement

Blood is to be collected from the cephalic vein of fasted dogs in serum separator tubes (Vacutainer SST, Becton Dickinson and Co., Franklin Lakes, NJ). The blood  
20 is centrifuged at 2000 rpm for 20 minutes and the serum decanted.

Total cholesterol may be measured in a 96 well format using a Wako enzymatic diagnostic kit (Cholesterol CII) (Wako Chemicals, Richmond, VA), utilizing the cholesterol  
25 oxidase reaction to produce hydrogen peroxide which is measured colorimetrically. A standard curve from 0.5 to 10  $\mu\text{g}$  cholesterol is to be prepared in the first 2 columns of the plate. The serum samples (20-40  $\mu\text{l}$ , depending on the expected lipid concentration) or known serum control  
30 samples are added to separate wells in duplicate. Water is added to bring the volume to 100  $\mu\text{l}$  in each well. A 100  $\mu\text{l}$  aliquot of color reagent is added to each well and

the plates will be read at 500 nm after a 15 minute incubation at 37 degrees centigrade.

HDL cholesterol may be assayed using Sigma kit No. 352-3 (Sigma Chemical Co., St. Louis, MO) which utilizes  
5 dextran sulfate and Mg ions to selectively precipitate LDL and VLDL. A volume of 150  $\mu$ l of each serum sample is to be added to individual microfuge tubes, followed by 15  $\mu$ l of HDL cholesterol reagent (Sigma 352-3). Samples are to be mixed and centrifuged at 5000 rpm for 5 minutes. A 50  
10  $\mu$ l aliquot of the supernatant is to be then mixed with 200  $\mu$ l of saline and assayed using the same procedure as for total cholesterol measurement.

Triglycerides are to be measured using Sigma kit No. 337 in a 96 well plate format. This procedure will  
15 measure glycerol, following its release by reaction of triglycerides with lipoprotein lipase. Standard solutions of glycerol (Sigma 339-11) ranging from 1 to 24  $\mu$ g are to be used to generate the standard curve. Serum samples

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(20-40  $\mu$ l, depending on the expected lipid concentration)  
20 are added to wells in duplicate. Water is added to bring the volume to 100  $\mu$ l in each well and 100  $\mu$ l of color reagent was also added to each well. After mixing and a 15 minute incubation, the plates will be read at 540 nm and the triglyceride values calculated from the standard  
25 curve. A replicate plate is also to be run using a blank enzyme reagent to correct for any endogenous glycerol in the serum samples.

#### **Dog Fecal Bile Acid Measurement**

30 Fecal samples may be collected to determine the fecal bile acid (FBA) concentration for each animal. Fecal collections may be made during the final 48 hours of the study, for two consecutive 24 hour periods between 9:00 am

and 10:00 am each day, prior to dosing and feeding. The separate two day collections from each animal are to be weighed, combined and homogenized with distilled water in a processor (Cuisinart) to generate a homogeneous slurry.

5 About 1.4 g of the homogenate is to be extracted in a final concentration of 50% tertiary butanol/distilled water (2:0.6) for 45 minutes in a 37°C water bath and centrifuged for 13 minutes at 2000 x g. The concentration of bile acids (mmoles/day) may be determined using a 96-

10 well enzymatic assay system (1,2). A 20 µl aliquot of the fecal extract is to be added to two sets each of triplicate wells in a 96-well assay plate. A standardized sodium taurocholate solution and a standardized fecal extract solution (previously made from pooled samples and

15 characterized for its bile acid concentration) will also analyzed for assay quality control. Twenty-microliter aliquots of sodium taurocholate, serially diluted to generate a standard curve are similarly to be added to two

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20 sets of triplicate wells. A 230 µl reaction mixture containing 1M hydrazine hydrate, 0.1 M pyrophosphate and 0.46 mg/ml NAD is to be added to each well. A 50 µl aliquot of 3a-hydroxysteroid dehydrogenase enzyme (HSD; 0.8 units/ml) or assay buffer (0.1 M sodium pyrophosphate) are then added to one of the two sets of triplicates. All

25 reagents may be obtained from Sigma Chemical Co., St. Louis, MO. Following 60 minutes of incubation at room temperature, the optical density at 340nm will be measured and the mean of each set of triplicate samples will be calculated. The difference in optical density  $\pm$  HSD

30 enzyme is to be used to determine the bile acid concentration (mM) of each sample based on the sodium taurocholate standard curve. The bile acid concentration of the extract, the weight of the fecal homogenate (grams)

and the body weight of the animal are to be used to calculate the corresponding FBA concentration in mmol/kg/day for each animal. The mean FBA concentration (mmol/kg/day) of the vehicle group is to be subtracted  
5 from the FBA concentration of each treatment group to determine the increase (delta value) in FBA concentration as a result of the treatment.

#### Intestinal Cholesterol Absorption Assay

10 A variety of compounds are shown to inhibit cholesterol absorption from the intestinal tract. These compounds lower serum cholesterol levels by reducing intestinal absorption of cholesterol from both exogenous sources (dietary cholesterol) and endogenous cholesterol  
15 (secreted by the gall bladder into the intestinal tract).

In hamsters the use of a dual-isotope plasma ratio method to measure intestinal cholesterol absorption has been refined and evaluated as described by Turley et al. (J. Lipid Res. 35, 329-339 (1994), herein incorporated by  
20 reference).

Male hamsters weighing 80-100 g are to be given food and water ad libitum in a room with 12 hour alternating periods of light and dark. Four hours into the light period, each hamster is administered first an intravenous  
25 dose of 2.5  $\mu$ Ci of [1,2- $^3$ H]cholesterol suspended in Intralipid (20%) and then an oral dose of [4- $^{14}$ C]cholesterol in an oil of medium chain triglycerides (MCT). The i.v. dose is given by injecting a 0.4 ml volume of the Intralipid mixture into the distal femoral vein.  
30 The oral dose is given by gavaging a 0.6 ml volume of the MCT oil mixture introduced intragastrically via a polyethylene tube. After 72 hours the hamsters are bled and the amount of  $^3$ H and  $^{14}$ C in the plasma and in the



original amount of label administered are determined by liquid scintillation spectrometry. The cholesterol absorption will be calculated based on the following equation:

5

Percent cholesterol absorbed =

$$\frac{\% \text{ of oral dose per ml of 72 hour plasma sample}}{\% \text{ of i.v. dose per ml of 72 hour plasma sample}} \times 100$$

10

#### Plasma Lipids Assay in Rabbits

Plasma lipids can be assayed using standard methods as reported by J.R. Schuh et al., J. Clin. Invest., 91, 1453-1458 (1993), herein incorporated by reference. Groups of male, New Zealand white rabbits are placed on a standard diet (100g/day) supplemented with 0.3% cholesterol and 2% corn oil (Zeigler Bothers, Inc., Gardners, PA). Water is available ad lib. Groups of control and treated animals are killed after 1 and 3 months of treatment. Tissues are removed for characterization of atherosclerotic lesions. Blood samples are to be taken for determination of plasma lipid concentrations.

25

#### Plasma Lipids

Plasma for lipid analysis is to be obtained by withdrawing blood from the ear vein into EDTA-containing tubes (Vacutainer; Becton Dickenson & Co., Rutherford, NJ), followed by centrifugal separation of the cells. Total cholesterol was determined enzymatically, using the cholesterol oxidase reaction (C.A. Allain et al., Clin. Chem., 20, 470-475 (1974), herein incorporated by reference). HDL cholesterol was also measured

enzymatically, after selective precipitation of LDL and VLDL by dextran sulfate with magnesium (G.R. Warnick et al., Clin. Chem., 28, 1379-1388 (1982), herein incorporated by reference). Plasma triglyceride levels  
5 will be determined by measuring the amount of glycerol released by lipoprotein lipase through an enzyme-linked assay (G. Bucolo et al., Clin. Chem., 19, 476-482 (1973), herein incorporated by reference).

#### 10        **Atherosclerosis**

Animals are to be killed by pentobarbital injection. Thoracic aortas are rapidly removed, immersion fixed in 10% neutral buffered formalin, and stained with oil red O (0.3%). After a single longitudinal incision along the  
15 wall opposite the arterial ostia, the vessels are pinned open for evaluation of the plaque area. The percent plaque coverage is determined from the values for the total area examined and the stained area, by threshold analysis using a true color image analyzer (Videometric  
20 150; American Innovision, Incl, San Diego, CA) interfaced to a color camera (Toshiba 3CCD) mounted on a dissecting microscope. Tissue cholesterol will be measured enzymatically as described, after extraction with a chloroform/methanol mixture (2:1) according to the method  
25 of Folch et al. (J. Biol. Chem., 226, 497-509 (1957), herein incorporated by reference).

#### **In Vitro Vascular Response**

The abdominal aortas are rapidly excised, after  
30 injection of sodium pentobarbital, and placed in oxygenated Krebs-bicarbonate buffer. After removal of perivascular tissue, 3-mm ring segments are cut, placed in a 37°C muscle bath containing Krebs-bicarbonate solution, and suspended between two stainless steel wires, one of

which is attached to a force transducer (Grass Instrument Co., Quincy, MA). Force changes in response to angiotensin II added to the bath will be recorded on a chart recorder.

5

In Vivo PPAR- $\alpha$  Assay for Fibric Acid Derivative Activity

The methods described by K. Murakami et al. (Diabetes, 47, 1841-1847 (1998) at 1842) for lipid metabolism and enzymatic activity of acyl-CoA oxidase in  
10 liver and Northern blotting can be used to assay in vivo for fibric acid derivative activity.

In Vitro Vascular Response

The abdominal aortas are to be rapidly excised, after  
15 injection of sodium pentobarbital, and placed in oxygenated Krebs-bicarbonate buffer. After removal of perivascular tissue, 3-mm ring segments are cut, placed in a 37°C muscle bath containing Krebs-bicarbonate solution, and suspended between two stainless steel wires, one of  
20 which is attached to a force transducer (Grass Instrument Co., Quincy, MA). Force changes in response to angiotensin II added to the bath will be recorded on a chart recorder.

25 The examples herein can be performed by substituting the generically or specifically described therapeutic compounds or inert ingredients for those used in the preceding examples.

The invention being thus described, it is apparent  
30 that the same can be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the present invention, and all such modifications and equivalents as would be obvious to one skilled in the art are intended to be included within the scope of the

following claims.

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CLAIMS

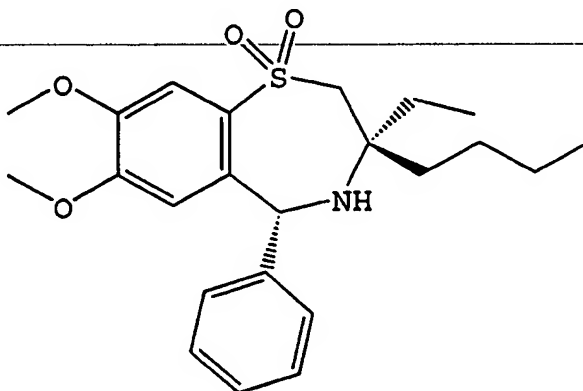
What is claimed is:

5

1. A therapeutic combination comprising a first amount of an ileal bile acid transport inhibiting compound and a second amount of a fibric acid derivative compound wherein the first amount and the second amount together comprise an anti-hyperlipidemic condition effective amount, an anti-atherosclerotic condition effective amount, or an anti-hypercholesterolemic condition effective amount of the compounds.

15

2. The therapeutic combination of claim 1 wherein the ileal bile acid transport inhibitor is a compound having the structure of formula B-2:

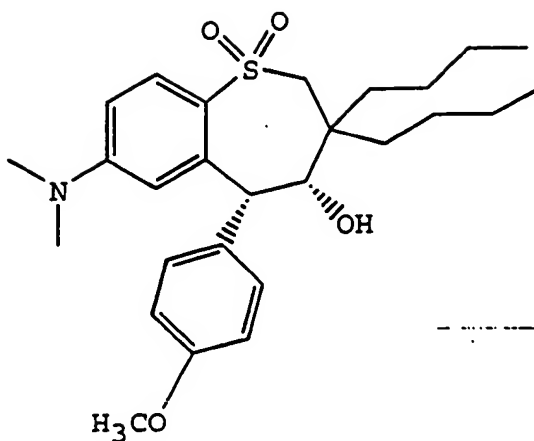


B-2

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or an enantiomer or racemate thereof.

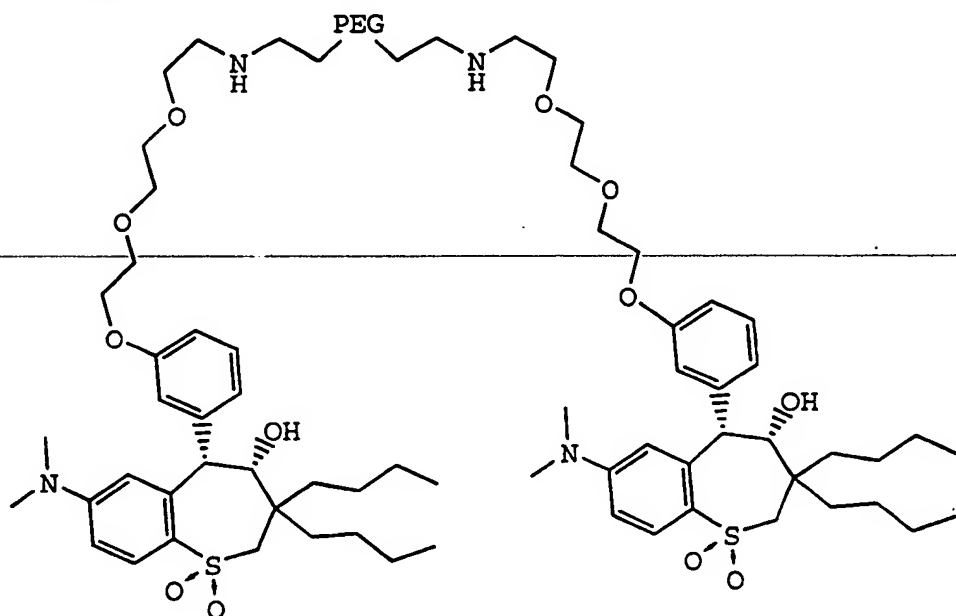
3. The therapeutic combination of claim 1 wherein the ileal bile acid transport inhibiting compound has the structure of formula B-12:



B-12

or an enantiomer or racemate thereof.

4. The therapeutic combination of claim 1 wherein the  
 5 ileal bile acid transport inhibiting compound has  
 the structure of formula B-29:

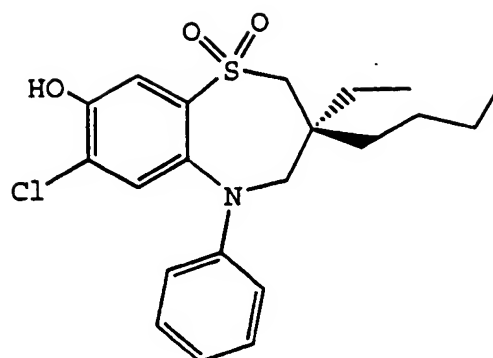


B-29

or an enantiomer or racemate thereof, wherein PEG  
 is an about 3000 to about 4000 molecular weight  
 polyethylene glycol polymer chain.

10

5. The therapeutic combination of claim 1 wherein the  
 ileal bile acid transport inhibiting compound has  
 the structure of formula B-7:



B-7

or an enantiomer or racemate thereof.

6. The therapeutic combination of claim 1 wherein the  
5 fibric acid derivative compound is clofibrate.
7. The therapeutic combination of claim 1 wherein the  
fibric acid derivative compound is gemfibrozil.
- 10 8. The therapeutic combination of claim 1 wherein the  
fibric acid derivative compound is fenofibrate.
9. The therapeutic combination of claim 1 wherein the  
15 fibric acid derivative compound is ciprofibrate.
10. The therapeutic combination of claim 1 wherein the  
fibric acid derivative compound is bezafibrate.
11. The therapeutic combination of claim 1 wherein the  
20 fibric acid derivative compound is clinofibrate.
12. The therapeutic combination of claim 1 wherein the  
fibric acid derivative compound is binifibrate.
- 25 13. The therapeutic combination of claim 1 wherein the  
combination comprises a composition comprising the  
ileal bile acid transport inhibiting compound and  
the fibric acid derivative compound.

14. A method for the prophylaxis or treatment of a hyperlipidemic condition comprising administering to a patient in need thereof a combination in unit dosage form wherein the combination comprises a first amount of an ileal bile acid transport inhibiting compound and a second amount of a fibric acid derivative compound wherein the first amount and the second amount together comprise an anti-hyperlipidemic condition effective amount of the compounds.
15. A method for the prophylaxis or treatment of an atherosclerotic condition comprising administering to a patient in need thereof a combination in unit dosage form wherein the combination comprises a first amount of an ileal bile acid transport inhibiting compound and a second amount of a fibric acid derivative compound wherein the first amount and the second amount together comprise an anti-atherosclerotic condition effective amount of the compounds.
16. A method for the prophylaxis or treatment of hypercholesterolemia comprising administering to a patient in need thereof a combination in unit dosage form wherein the combination comprises a first amount of an ileal bile acid transport inhibiting compound and a second amount of a fibric acid derivative compound wherein the first amount and the second amount together comprise an anti-hypercholesterolemic condition effective amount of the compounds.



PCT/US 99/27948

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 7      A61K45/06      A61K31/55      A61P9/00

IPC 7 A61K

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

A	WO 94 09774 A (MERCK) 11 May 1994 (1994-05-11) claims 5,6,9 page 16, line 6 -page 17, line 2	1,6,7,13
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**Y** Patent family members are listed in annex.

"&" document member of the same patent family

Peeters, J

# INTERNATIONAL SEARCH REPORT

Internat' Application No  
PCT/US 99/27948

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9409774 A	11-05-1994	US 5256689 A AU 5538794 A	26-10-1993 24-05-1994

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